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The Relationship of Stable Fly Abundance to Milk Production in Dairy Cattle[1]

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Abstract

Intensive studies, involving two distinct procedures, conducted over a period of 3 years (1955–57) developed very significant correlations between stable fly (*Stomoxys calcitrans* (L.)) abundance and reductions in milk and butterfat production during the summer months (May-September). On the basis of two distinct regression lines, average monthly rates of loss were established as 0.65 and 0.7% per fly per cow. Evidence is presented indicating that depressed production continues for weeks or months beyond the end of the fly season. Thus good stable fly control for 4 months may produce significant benefits for 6 months or longer.

The contents of this article represent the results of 3 years of intensive studies on the effect of biting flies upon the productivity of dairy cattle.

Freeborn *et al.* (1925) found that stable flies caused 9.26% and horn flies 3.33% loss in milk production. Experiments by a number of investigators (Bruce & Decker 1947, 1951, Decker 1955, Granett & Hansens 1956, 1957, Cutkomp & Harvey 1958, and others) reveal similar trends toward a loss of productivity of milk and meat which could be attributed to the presence of biting flies. Most of these studies were concerned mainly with the short-term effects of fly attacks and some involving two- to four-month periods were primarily concerned with the effect of horn flies (*Siphona irritans* (L.)) on beef production. None of the authors mentioned attempted to develop a correlation between numbers of biting flies and losses incurred, and except for Bruce and Decker none developed information with regard to attrition resulting from prolonged exposure to biting flies.

This study represents an attempt to bring together correlative data on milk productivity and stable fly population densities and develop further evidence on the attrition factor.

After several seasons of work designed to evaluate various repellent formulations used upon dairy cattle to control biting flies, experiments were developed to measure the practical benefits of fly control. Our interest in this study was not concerned with obtaining maximum levels

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Table 1.—Butterfat production gradients (May = 100%) and the average number of stable flies per cow on treated and check animals within the same herd from May 1 to September 30, 1955, 1956, and 1957.

Herd	Year	Treatment	No.[a] Cows Used	Production Gradient: Per Cent of Initial (May) Monthly Production of Butterfat — May	June	July	Aug.	Sept.	Four Month Average	Average Per Cent Loss	Average No. Flies per Cow[b]
A	1955	Mediocre	7	100	75.6	75.0	72.0	73.0	73.9	26.1	6.4
B	1955	Poor	6	100	76.1	72.7	71.4	68.5	72.2	27.8	14.5
A	1956	Good	4	100	88.3	90.3	69.5	62.5	77.6	22.4	1.1
B	1956	Good	7	100	86.7	73.9	71.5	65.5	74.1	25.9	1.6
A	1957	Mediocre	7	100	78.7	67.2	63.5	57.1	66.6	33.4	8.4
B	1957	Mediocre	4	100	73.9	67.6	74.9	67.4	70.5	29.5	5.3
A	1955	Check[c]	5	100	80.5	68.0	62.1	57.7	67.1	32.9	17.0
B	1955	Check[c]	6	100	75.3	69.3	62.5	64.3	67.8	32.2	19.0
A	1956	Check[c]	4	100	80.5	69.6	57.5	47.0	63.9	36.1	24.6
B	1956	Check[c]	6	100	82.7	67.5	63.0	56.4	67.4	32.6	18.5
A	1957	Check[c]	7	100	67.8	52.0	45.7	41.1	51.3	48.7	38.8
B	1957	Check[c]	7	100	67.3	64.4	56.1	50.5	57.7	42.3	31.3

[a] All cows in continuous lactation. Mean differences for significance in production gradients are: 5% level = 6.52%; 1% level = 8.73%.

[b] Stable flies were counted on cows 5 days each week at 11 A.M. and 3 P.M. Correlation coefficient between average number of stable flies and average accumulative loss in butterfat production: r = 0.645. Required for significance: 5% level = .576; 1% level = .703.

[c] Check animals received some temporary benefits from space spray used in barn which contained 0.15% pyrethrins and 0.75% activator.

Table 2.—Average butterfat gradient (departures from May—100%) in treated and check halves of two herds for 4 months (June, July, August, and September) of 1955, 1956, and 1957.

Year	Herd	No. of Cows[a]		Butterfat Gradient[b]			Per Cent Increase, Treated Over Check
				Average Monthly (June, July, Aug., Sept.) Per Cent of May (100%) Butterfat Production			
		Treated	Check	Treated	Check	Difference	
1955	A	7	5	73.9	67.1	6.8	10.1
	B	6	6	72.2	67.8	4.4	6.5
1956	A	4	4	77.6	63.9	13.7	21.4
	B	7	6	74.1	67.4	6.7	9.9
1957	A	7	7	66.6	51.3	15.3	29.8
	B	4	7	70.5	57.7	12.8	22.2

[a] All cows used in this study were in continuous lactation.
[b] Difference between the treated and check animals reveals gain as a result of fly control. Mean difference for significance: 5% level = 6.52%; 1% level = 8.73%.

of fly control, but rather in the development of information with respect to the value of various levels of fly control on dairy cattle. The data so developed were used to construct regression curves and obtain a correlation coefficient between the number of flies found upon cattle and the amount of milk produced. Such a correlation coefficient should be of practical value to those wishing to make reasonable estimates of the extent of milk losses where data are available on stable fly abundance.

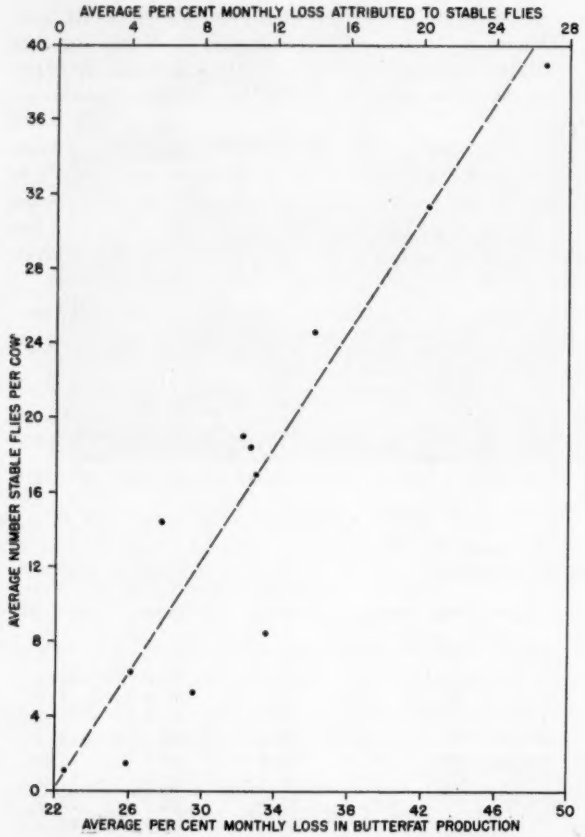

FIG. 1.—Regression line showing the relationship between average numbers of stable flies occurring on the treated and untreated halves of two herds and the butterfat-loss gradients from May through September for 1955, 1956, and 1957. The losses indicate average monthly percentage drop in production from the production for the month of May.

Table 3.—Average per cent difference in butterfat production between treated and check halves of two herds, A and B, by months for 1955, 1956, and 1957 and the average numbers of stable flies for the same period.

	May	June	July	August	September
Average per cent increase of treated animals over check animals	0.0	5.3	12.6[a]	18.0[a]	23.3[a]
Average no. stable flies per cow:					
Treated animals	9.5	5.6	6.3	6.4	4.5
Check animals	9.6	17.9	24.7	27.4	29.3

[a] Significant at both the 1% and 5% levels.

METHOD OF STUDY.—Three years (1955–1957) of experimentation with spray formulations giving various levels of fly control have given the desired information on lactation gradients for individual animals in several herds.

Two methods of study were employed to obtain the data. One was the split-herd treatment in which one-half of the animals received a daily application of an effective

Table 4.—Milk production gradients of animals in continuous lactation, with the average number of stable flies found upon cattle among various herds for 1955, 1956, and 1957.

Herd	Year	No. of Animals in Continuous Lactation	Per Cent Active Ingredients in Fly Spray		Production-Loss Gradient[a,b]	Average Number of Stable Flies per Cow from June 1 to Sept. 30
I	1955	7	0.2	pyrethrins		
			2.0	piperonyl butoxide	21.4	8.9
II	1955	16	0.2	pyrethrins		
			2.0	R-326[c]		
			2.0	MGK-264	13.7	2.3
III	1955	5	0.2	pyrethrins		
			2.0	piperonyl butoxide	20.2	9.1
IV	1955	4	0.1	pyrethrins		
			1.0	R-326		
			1.0	MGK-264	20.5	6.7
V	1955	21		As above	20.4	4.5
VI	1955	7		None	29.1	25.5
VII	1955	11	0.5	piperonyl butoxide		
			0.05	pyrethrins	25.6	14.1
VIII	1956	6	0.5	piperonyl butoxide		
			0.05	pyrethrins	24.6	12.3
IX	1956	5	0.35	piperonyl butoxide		
			0.035	pyrethrins	32.9	16.0
X	1956	15	0.05	pyrethrins		
			1.0	Tabutrex	23.8	6.5
XI	1956	9		None	35.5	28.7
XII	1956	15	0.05	pyrethrins		
			2.0	Tabutrex[d]	20.7	3.2
XIII	1957	8	0.05	pyrethrins		
			0.5	Tabutrex	16.0	3.6
XIV	1957	5	0.35	piperonyl butoxide		
			0.035	pyrethrins	26.1	13.4
XV	1957	13	0.05	pyrethrins		
			0.5	Tabutrex	19.6	5.4
XVI	1957	8		None	34.1	25.6
XVII	1957	3		None	42.2	36.3
XVIII	1957	4		None	36.6	26.7

[a] Production-loss gradient = 100 − average (4 mo.) production gradient (% of June 100), June 1 to October 31.
[b] Mean difference for significance among production gradient of herds: 5% level = 8.43%; 1% level = 11.25%.
Correlation coefficient $r = .898$; required for significance, 5% level = .468; 1% level = .590.
[c] Di-*n*-propyl isocinchomeronate.
[d] Dibutyl succinate.

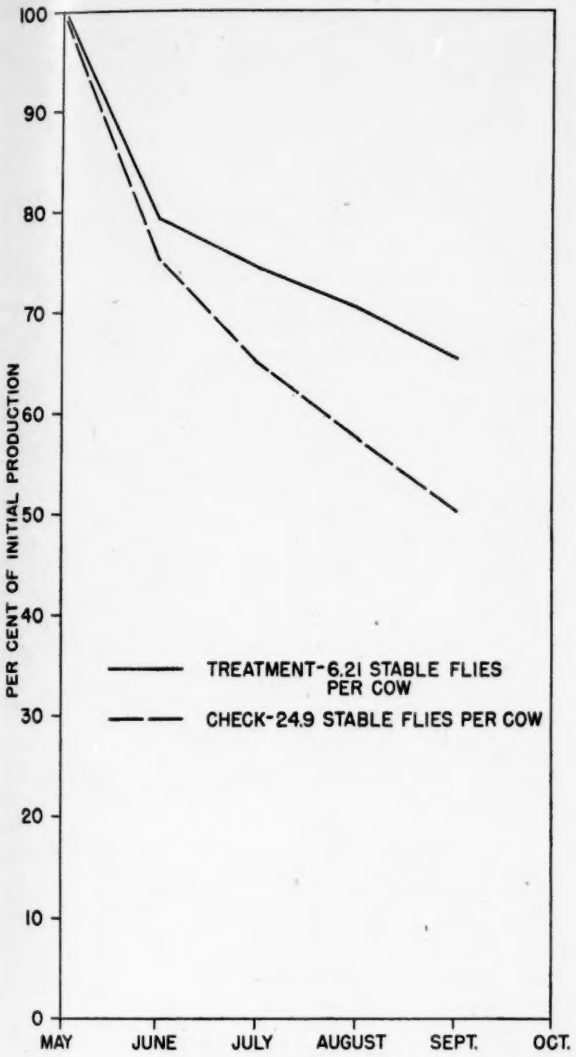

FIG. 2.—Average per cent of initial butterfat production (May) for the treated and untreated halves of two herds from May through September during 1955, 1956, and 1957. The untreated animals became progressively less productive than the treated animals through the course of the summer.

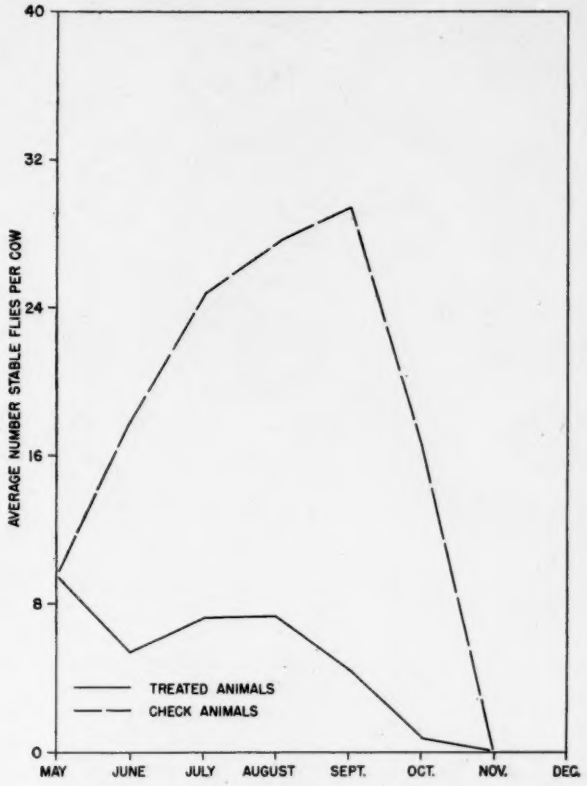

FIG. 3.—Average numbers of stable flies per cow occurring on the treated and untreated halves of two herds during the summers of 1955, 1956, and 1957. The stable fly population on the untreated animals shows the typical trend in fly counts for the period of May through October.

repellent spray mixture and the other half served as an untreated check. Comparisons of productivity were made between individual animals in the two halves of the herd. Daily milk and butterfat production figures were kept on each animal under study in order to calculate the butterfat gradients. The advantage of the split-herd treatment was that the variables that might arise from herd management, such as pasturing, supplementary feeds, etc., were eliminated.

The second method of study involved the treatment of whole herds with repellent sprays so selected as to obtain different levels of fly control. These tests were carried out on well-managed dairy farms where adequate records of milk production could be obtained easily. Throughout the 3 years (1955–1957) a total of 18 dairy herds were used in the whole-herd experiments.

In both experiments the animals employed were in similar stages of lactation. Animals that had just freshened or those that had been in lactation too long (4 to 5 months) were not used in the experiments. Consequently the lactation or butterfat gradients exhibited by the cows were regressing normally when the tests commenced. Since the rate at which productivity declined from one month to the next was altered by variations in the pressure applied by biting flies, mean monthly lactation gradients proved to be measurements which could be correlated with the average number of flies observed on the animals.

The first month's (May or June, as indicated) production of milk or butterfat was used as a base from which the production for succeeding months could be expressed as a percentage. This reduced the inherent differences in productivity due to breeds or breeding to a common denominator which permits comparisons. Thirty-one-day monthly production figures were corrected to 30 days to obtain the average amount of milk or butterfat per animal for each month.

Actually the methods of application or materials used in these tests are unimportant, since the objective was to obtain various levels of fly control and not an optimum degree of control. All sprays were applied with hand-operated sprayers at the usual daily dosage of 2 ounces per animal. Treatments were started June 13, 11, and 10 for years 1955, 1956, and 1957, respectively.

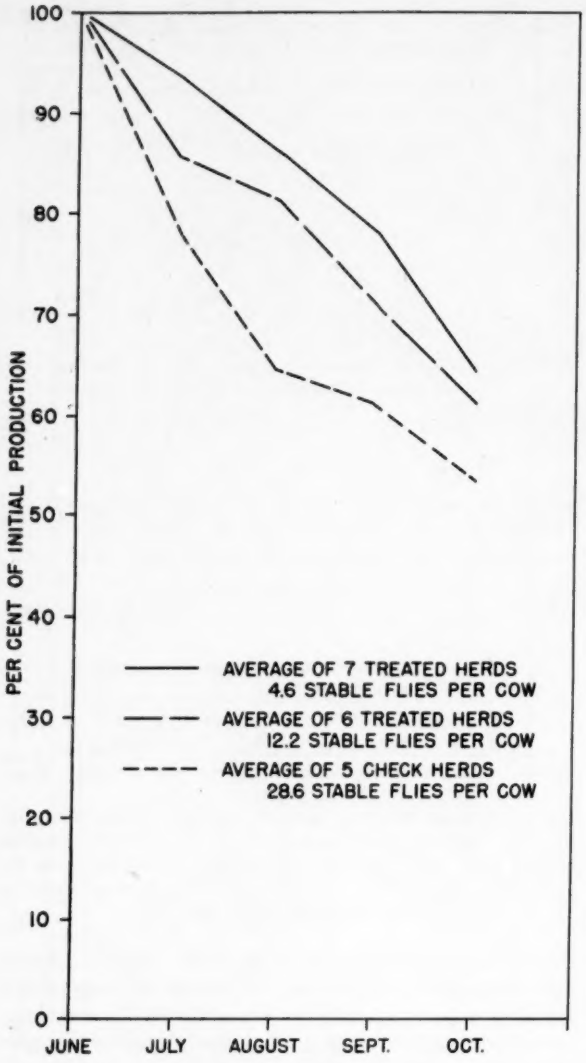

FIG. 4.—Average per cent of initial milk production for animals in similar stage of lactation. The three gradients represent the averages of herds which had low, intermediate, and high incidence of stable flies present during the summer. Thirteen of the eighteen herds were treated with repellent sprays to reduce the stable fly population to various levels.

Horn flies were not present in sufficient numbers on the various herds to justify tabulations on their presence. They usually disappeared 7 to 10 days after the daily application of spray was started. To eliminate horn flies on the check animals, spray was permitted on the animals not involved in production data. Horse flies, *Tabanus sulcifrons* Macq., were present for a short period of time (4 to 6 weeks) during June and July on herds VI, XI, and XVI. It is doubtful that their presence at a population density of three to five horse flies per cow would significantly alter the results of the total production trend for the 5-month period. Stable fly counts were made three times per week between 10 A.M. and 4 P.M. on the 18 herds in the whole-herd tests. In the case of the split-herd experiment, fly counts were made at 11 A.M. and 3 P.M., 5 days of each week.

RESULTS.—Productivity and correlative data from the split-herd treatments are both interesting and conclusive in nature. Tables 1 and 2 contain a summary of the analyzed data. Accordingly, there is a significant degree of correlation between the average number of stable flies per cow and the butterfat gradients. The correlative relationship existing between a dairy cow's productivity and stable fly abundance is illustrated in figure 1. There is also a significant difference between the treated and untreated halves of each herd within each year of study. During the period May 1–September 30, there appears to be a natural 22% reduction in butterfat production not attributable to flies, and when we subtract 22% from the average monthly gradients, we obtain values for average losses attributable to flies ranging from 1 to 26%. Computations based on the regression line shown in figure 1 indicate an average production loss of 0.65% per fly per cow. There was also a highly significant difference between the butterfat gradients for the months of July, August, and September for the treated and untreated animals, table 3.

When the average production trends of the treated animals and the check animals are graphed, figure 2, one immediately notices an ever-increasing difference in

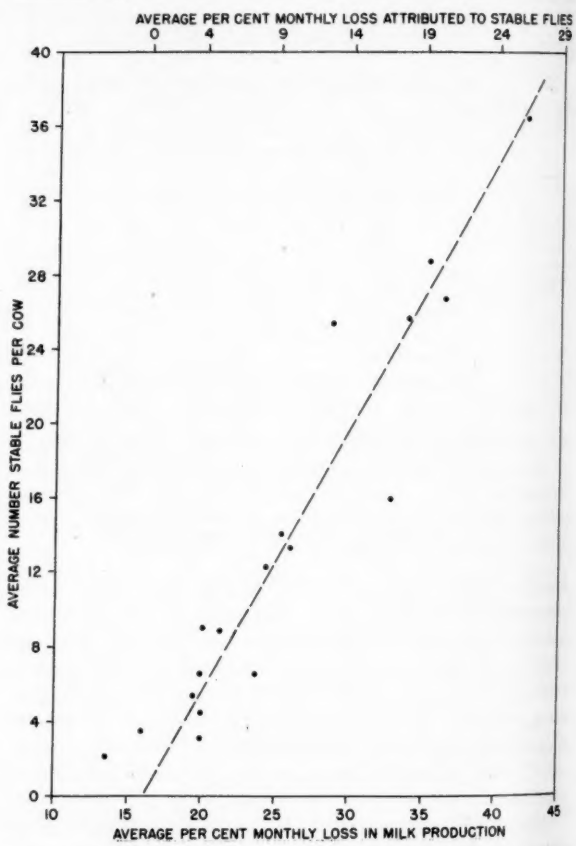

FIG. 5.—Regression line showing the relationship between average numbers of stable flies occurring on herds and their 4-month average lactation-loss gradients. The greatest losses occur with the herds having the highest average number of stable flies during the summer. The losses indicate average monthly percentage drops in production from the base production for the month of June.

butterfat production occurring with each succeeding period of time. This represents the long-term effect of continued attack by biting flies which is apparently attributable to attrition. The average number of stable flies observed on the treated and check animals of the split-herd treatments is shown by months for the 3 years, 1955–1957, in figure 3. These data show that the number of flies on treated animals declined steadily after the first spray was applied in May, whereas populations on untreated animals rose to a peak in September.

Results from the whole herd treatments summarized in table 4 show the data on milk production of 18 herds and the average numbers of stable flies per cow for the entire test period. A close correlation (R = .898) exists between the incidence of stable flies and the rate at which lactation declines. Furthermore, the average differences among the milk production gradients are significant as derived by an analysis of variance of monthly production records. At the 5% level, 8.43% is a significant difference, and a difference of 11.25% is significant at the 1% level. A grouping of the lactation data by months according to high, intermediate, and low levels of fly populations produces three distinct lactation curves as shown in figure 4. Of special interest is the fact that the differences in lactation are observable through the month of October, even though most of the flies had disappeared. This may be attributed to the fact that once milk secretion has been materially reduced by attrition, the process cannot ordinarily be reversed until the beginning of the next lactation period.

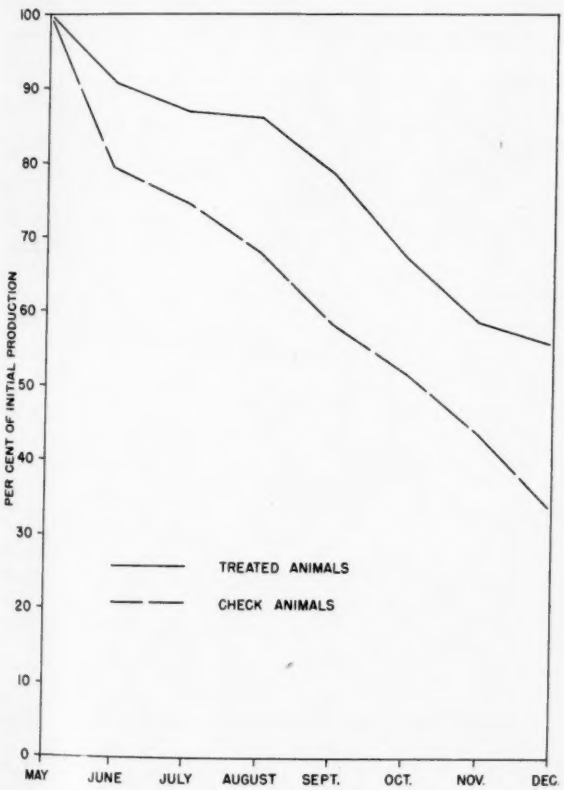

FIG. 6.—The average per cent of initial butterfat production for treated and untreated animals within the same herd from May through December, 1956. The treated animals remained at a higher level of production during October, November, and December even though flies were no longer present.

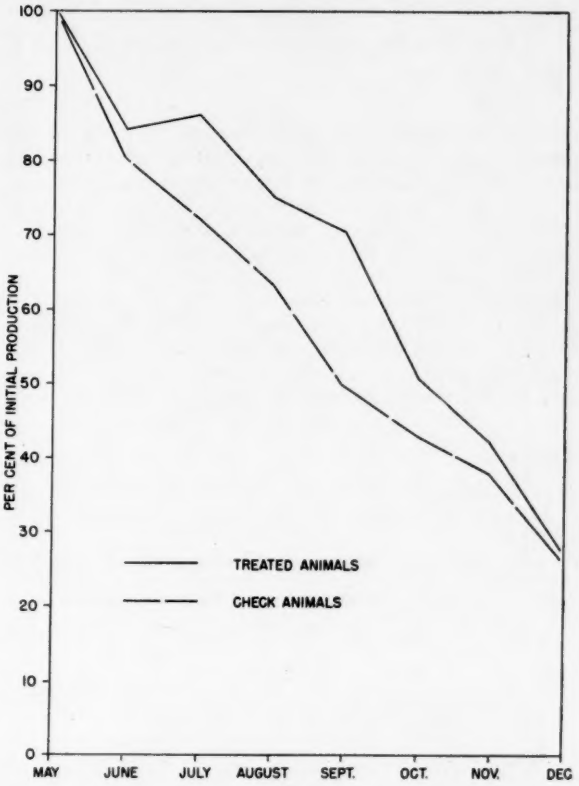

FIG. 7.—Average per cent of initial milk production for treated and untreated animals within the same herd from May through December. Beneficial effects of fly control are evident beyond the season of fly activity.

Calculations made from the regression curve, figure 5, indicate an average production loss of 0.7% per fly per cow. This is remarkably close to and fully substantiates the 0.65% value obtained for butterfat in figure 1.

The condition referred to as attrition as applied to productivity of dairy cows is the devitalizing effect of persistent attacks by biting flies throughout the summer. It may be attributed to annoyance of pain, blood losses, interference with normal grazing habits, possibly anaphylaxis from fly-derived substances left in the animal, or energy consumed by the animal in its efforts to ward off the flies. Apparently this represents a physiological phenomenon. Fortunately some information on long-term effects of fly attack have been accumulated. Figure 6, which presents average production records of individual animals of both treated and check cows in Herd B, 1956, shows that the decreased productivity of the summer months was sustained through December or long after the flies had disappeared. Furthermore, it was noted that, in general, the treated animals of the split-herd experiments had longer lactation periods and were out of production for a correspondingly shorter period of time than the untreated cows.

In another split-herd experiment on a local dairy farm, similar but less pronounced trends were revealed upon

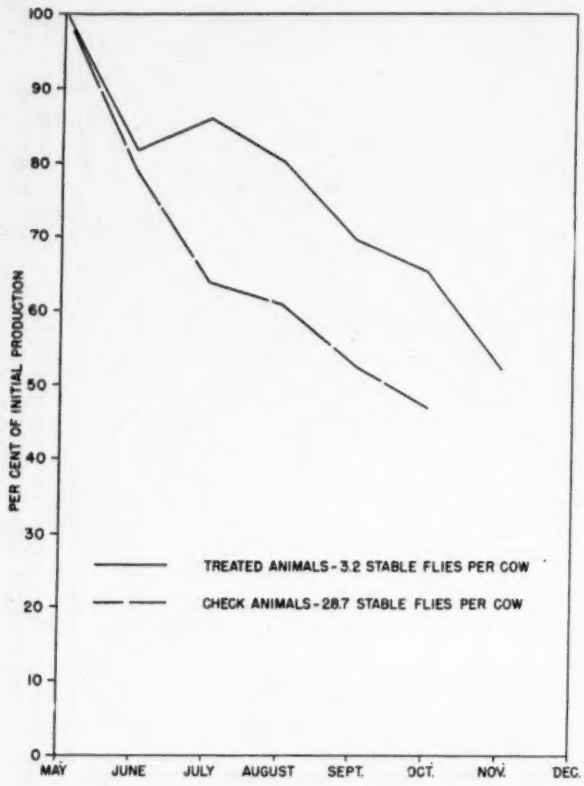

FIG. 8.—The average per cent of initial milk production for animals in a treated and untreated herd. The animals in the treated herd remained in production longer than those in the untreated herd. *Tabanus sulcifrons* Macq. were found on the untreated animals during the last 2 weeks of June and July.

examination of the production records, figure 7.

A comparison of individual animal production records of two separate herds in southern Illinois for the period of May to October displays a more striking difference in their lactation gradients which persists through October, figure 8. The animals in the treated herd remained in production 6 weeks longer than the untreated animals. This latter comparison was made between one herd which enjoyed the maximum level of fly control and another on which no control measures were used.

The deleterious effects of biting flies appear to be far-reaching in causing losses in the production of milk; not limited to the period of attack, but sustained and even accumulative in effect upon the vitality of the animals.

There is little doubt that effective control of flies upon cattle is rewarded by greater net returns for the herdsman, especially when he takes into account the immediate and latent results. A mathematical estimate indicates that a 1% increase in production for 6 months is required to pay for the spray material. Since the numerical values on increased production resulting from fly control, in the range of population densities described in this paper, suggest that increases of 10 to 20% should result from the proper application of good repellent spray formulations, there seems to be no room for doubt that good control of biting flies can and will pay handsome dividends.

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Taeniothrips orionis Leh., A Thrips Destructive to Vegetables in Alaska[1]

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In the summer of 1954 a thrips identified as *Taeniothrips orionis* Leh.[2] severely injured lettuce in Alaska near Soldotna, Kenai and Naptowne on the Kenai peninsula and potatoes near Kenai. Injury was severe enough to affect the marketability of lettuce. Infestation on potatoes was controlled by DDT. No damage was apparent in 1955 and 1956 that could be attributed to thrips.

In August 1957 this same thrips severely injured cabbage, lettuce and potatoes near Kenai. This is in the same general area as found infested in 1954. This appears to be an example of a native insect changing its food habits when cultivated crops are introduced into a newly cleared area. Previous records of this species indicate that its main hosts are wild plants, mountain lupine in British Columbia, Treherne (1924) and columbine, Herrick (1925). These are believed to be the first records of this species injuring cultivated crops.

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[1] Journal Series No. 17. Alaska Agricultural Experiment Station. Accepted for publication March 17, 1958.

[2] Determined by Miss Kellie O'Neil, U. S. Department of Agriculture, Entomology Research Division, Insect Identification and Parasite Introduction Section.

The Effect of Biting Fly Control on Weight Gain in Beef Cattle[1]

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ABSTRACT

During the summer of 1957, experiments to determine the effect of biting fly control on weight gain in beef cattle were conducted in Centre County, Pennsylvania. Insecticide-repellent formulations were applied daily to a herd of Aberdeen Angus by means of an electric-eye-controlled sprayer at the rate of approximately 75 ml. per animal. A water-base spray containing N,N-diethyl-m-toluamide and methoxychlor provided effective protection against biting flies, which included the horn fly, *Siphona irritans* (L.), the stable fly, *Stomoxys calcitrans* (L.), and a negligible number of a horse fly, *Tabanus quinquevittata* (Wied.). Equally satisfactory results were achieved with an oil-base spray containing 2,3,4,5-bis (Δ_2 butylene)-tetrahydrofurfural, pyrethrins, N-octyl bicycloheptene dicarboximide and piperonyl butoxide. Both formulations were much more effective against the horn fly than against the stable fly, when data for each species were treated separately. After 8 weeks of treatment, none of the cattle was adversely affected.

The favorable effect of biting fly control was demonstrated by the greater weight gain of the treated animals as compared with that of the untreated. As a result of treatments, the mean gain in weight of the treated group was found to surpass that of the untreated group by approximately one-half to two-thirds of a pound per animal per day. Statistical analyses indicated that the difference in weight gain between the two groups was significant. The cost of spray material was, in average, one cent a day for each animal.

Various estimates of the effect of biting fly control on beef production have been made but actual experimental data are relatively limited. Laake (1946) reported that, as a result of applying DDT on beef cattle in Kansas for the control of horn flies, the mean weight gain of treated animals surpassed that of the untreated by 42 to 70 pounds in 2 to 3 months. The same author estimated the loss due to injury by horn flies in Kansas alone in 1945 to be about $10,000,000. The investigations reported in

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Partial cost of publication of this paper was met by McLaughlin-Gormley-King Co., Minneapolis, Minnesota, to secure early publication.

[2] Professor of Zoology.

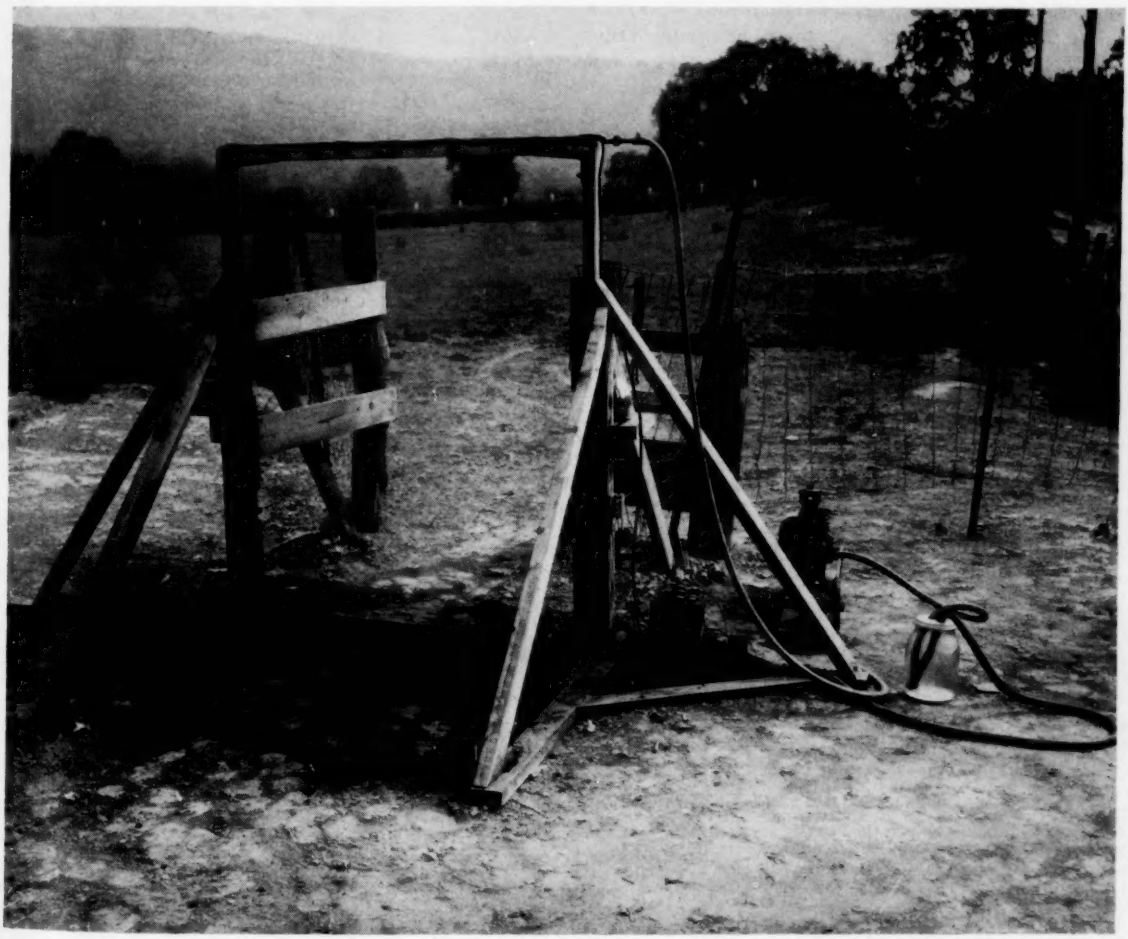

FIG. 1.—The electric-eye-controlled sprayer mounted at a chute placed near a fence.

this paper were designed to demonstrate the value of fly control and to compare the efficacy of recently introduced materials against biting flies on cattle.

EXPERIMENTAL PROCEDURE.—The experiments were conducted during the summer of 1957 on the Bennett Farm, Centre County, Pennsylvania.[3] The biting flies in the area consisted primarily of the horn fly, *Siphona irritans* (L.), and the stable fly, *Stomoxys calcitrans* (L.). A small number of a horse fly, *Tabanus quinquevittata* (Wied.), occurred for a brief period. Before spraying experiments began, 59 Aberdeen Angus in a herd were weighed three times in as many days to determine the mean initial weight of each animal. The animals were then carefully screened and assigned to treated and untreated groups in such a way that the mean body weight, age and general physical condition of the two groups were closely similar. The treated group consisted of 35 animals and the untreated of 24. Fly population counts taken during pretreatment periods showed no significant difference in the degree of infestation between the two groups. The treated and untreated groups were kept on opposite sides of a 45-acre pasture divided by two fences which were 10 feet apart. Both used similar grazing grounds and were given the same supplementary feed.

At one side of the pasture, where the treated animals were located, an electric-eye-controlled sprayer (Cheng *et al.* 1957) was installed at a chute placed next to a fence (fig. 1). A 6-volt battery supplied electric power for the photoelectric relay unit, and a 1-horsepower gasoline engine operated the pump. The following insecticide-repellent formulations were applied to the treated group by means of the sprayer: I. 15% N,N-diethyl-m-toluamide and 1% methoxychlor in water emulsion; II. 0.4% MGK Repellent 11 [2,3,4,5-bis (Δ_2 butylene)-tetrahydrofurfural], 0.2% MGK 264 (N-octyl bicycloheptene dicarboximide, insecticide synergist), 0.1% piperonyl butoxide and 0.035% pyrethrins in petroleum distillate.

Treatments were applied once a day, in the morning. The amount of spray delivered averaged approximately 75 ml. per animal. As a rule, five applications were made per week, except on several occasions when treatments had to be suspended because of unfavorable weather or change of spray material. The horn, stable and horse flies were counted with the aid of field glasses at 6 and 24 hours after spraying. The mean number of flies per animal was determined by counting the flies on any 10 animals in each of the treated and untreated groups.

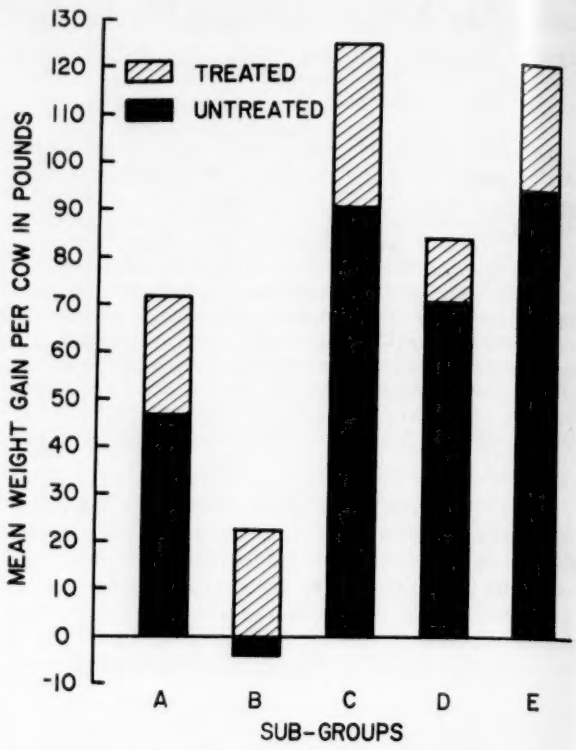

FIG. 2.—Weight gain of different sub-groups of treated and untreated beef cattle over a period of 8 weeks. (Sub-groups A and B: cows with calves before and after spray treatments began, respectively; C: cows 3 years of age and above; D and E: 2- and 1-year-old heifers, respectively.)

Table 1.—The effectiveness of insecticide-repellent formulations applied with an electric-eye-controlled sprayer[a] against horn flies and stable flies on beef cattle. Bennett Farm, Centre County, Pennsylvania. July–August, 1957.

FORMULATIONS AND INGREDIENTS	PERIODS OF TREATMENT (WEEKS)	MEAN NO. FLIES PER COW PER DAY[b]		PER CENT CONTROL[c]
		Treated	Untreated	
I				
15% N,N-diethyl-m-toluamide	1st	3.0	35.0	91.3
	2nd	3.6	28.8	87.5
1% Methoxychlor (in water emulsion)	3rd	4.6	34.3	86.6
	4th[d]	—	—	—
II				
0.400% MGK Repellent 11[e]	5th	3.8	81.2	95.3
0.200% MGK 264[f]	6th	9.9	69.6	85.8
0.100% piperonyl butoxide	7th	14.3	94.3	84.8
0.035% pyrethrins (in petroleum distillate)	8th	17.1	109.4	84.4

[a] At the rate of 75 ml. per cow.
[b] Flies were counted at both 6 and 24 hours after spraying on 35 treated Aberdeen Angus and 24 untreated.
[c] t-test for difference in effectiveness between formulations I and II: t = 0.3008 (not significant).
[d] Treatments suspended in order to change spray material.
[e] 2, 3, 4, 5-bis (Δ_2 butylene)-tetrahydrofurfural.
[f] N-octyl bicycloheptene dicarboximide, insecticide synergist.

Results and Discussion.—Results of the experiments on the effectiveness of the formulations are shown in table 1. Over a period of 3 weeks, the per cent control of the horn and stable flies achieved by formulation I, a water-base spray containing N,N-diethyl-m-toluamide and methoxychlor, varied from 86.6 to 91.3, with a mean of 88.5. Formulation II, an oil-base spray containing MGK Repellent 11, pyrethrins, MGK 264 and piperonyl butoxide, provided from 84.4 to 95.3% control of the flies, with a mean of 87.6% during a 4-week period. As the principal ingredients in the formulations were not tested separately, the exact role played by each in fly control remains to be determined. The difference in effectiveness

[3] Grateful acknowledgment is made to Dr. K. R. Bennett of the Department of Agricultural Economics and Rural Sociology for his close cooperation which has made this study possible. Chemicals were generously donated by McLaughlin-Gormley-King Co., Minneapolis, Minnesota, and Hercules Powder Co., Wilmington, Delaware.

Table 2.—Gains in weight of cows treated with insecticide-repellent formulations for the control of biting flies[a] as compared with weight of untreated animals. Bennett Farm, Centre County, Pennsylvania. July–August, 1957.

Treated Group					Untreated Group				
		Weight (Pounds)					Weight (Pounds)		
Tag No.	Age (Years)	Initial	Final	Gain or Loss	Tag No.	Age (Years)	Initial	Final	Gain or Loss
				A. Cows with calves before treatments began (May 20 to June 5)					
1	6	800	907	107	1	9	928	956	28
2	13	1025	1072	47	2	5	956	1018	62
3	11	944	1023	79	3	7	806	878	72
4	8	844	946	102	4	5	769	843	74
5	8	846	947	101	5	7	1012	1020	8
6	3	600	654	54	6	8	831	872	41
7	4	871	910	39					
8	5	855	1006	151					
9	2	786	755	−31					
Total	60	7571	8220	649		41	5302	5587	285
Mean	6.7	841.2	913.3	72.1		6.8	883.7	931.2	47.5
				B. Cows with calves after treatments began (July 5 to August 15)					
10	5	963	986	23	7	4	685	693	8
11	7	1163	1187	24	8	7	1036	1063	27
					9	6	948	943	−5
					10	9	1119	1071	−48
Total	12	2126	2173	47		26	3788	3770	−18
Mean	6	1063	1086.5	23.5		6.5	947	942.5	−4.5
				C. Cows without calves					
12	7	999	1154	155	11	9	875	972	97
13	5	754	965	211	12	5	900	1023	123
14	4	913	1028	115	13	4	834	900	66
15	4	931	946	15	14	3	580	658	78
16	3	721	845	124					
17	3	708	825	117					
18	3	631	706	75					
19	3	692	835	143					
20	3	650	823	173					
Total	35	6999	8127	1128		21	3189	3553	364
Mean	3.9	777.7	903.0	125.3		5.2	797.2	888.2	91.0
				D. Two-year-old heifers					
21	2	663	750	87	15	2	621	691	70
22		705	762	57	16		686	724	38
23		567	639	72	17		708	814	106
24		609	729	120					
Total		2544	2880	336			2015	2229	214
Mean	2	636	720	84		2	671.7	743	71.3
				E. One-year-old heifers					
25	1	495	600	105	18	1	529	608	79
26		447	618	171	19		376	501	125
27		459	571	112	20		423	519	96
28		592	696	104	21		494	563	69
29		595	794	199	22		485	588	103
30		516	634	118	23		433	521	88
31		472	622	150	24		315	419	104
32		452	509	57					
33		462	591	129					
34		519	607	88					
35		597	707	110					
Total		5606	6949	1343			3055	3719	664
Mean	1	509.6	631.7	122.1		1	436.4	531.3	94.9
Grand total	126	24846	28349	3503		101	17349	18858	1509
Overall mean per cow	3.6	709.88	809.97	100.09		4.2	722.87	785.75	62.88

[a] Primarily horn flies and stable flies.

between the two formulations was not significant. Both were much more effective against the horn fly than against the stable fly, when the data for each species were treated separately. Since the horse fly occurred in very small numbers, it was not included in the data. None of the cattle was adversely affected by the treatments.

The effect of biting fly control on weight gain was indicated by the difference between initial and final weights of treated and untreated animals during the 8-week experimental period. As shown in table 2, the body weight data of both groups of cows were classified under five sub-groups for each of the treated and untreated groups. Cows calving several weeks before and after treatments began were placed in sub-group A and B, respectively; cows, 3 years of age and above, in sub-group C; 2-year-old heifers in sub-group D; and 1-year-old heifers in sub-group E.

During the 8-week period, the overall mean weight gain per cow, without reference to the sub-groups, was 100.09 pounds for the treated group and 62.88 pounds for the untreated. The t-test showed that the overall difference of 37.2 pounds per cow was significant at the 0.01 level ($t=2.965$).

The overall difference mentioned above was probably biased upwards by the disproportionate numbers of animals in sub-group B, With the latter sub-group removed, the overall difference was 28.4 pounds per cow, which was significant at the 0.05 level ($t=2.259$).

The mean weight gains in the five sub-groups of treated animals were greater than those of the untreated by 24.6 pounds per cow in sub-group A, 28.0 pounds in sub-group B, 34.3 pounds in sub-group C, 12.7 pounds in sub-group D, and 27.2 pounds in sub-group E (fig. 2). With weights assigned to different sub-groups, the average difference was 26.2 pounds per cow. The t-test indicated that the difference was significant at the 0.02 level ($t=2.47$).

The above analyses show that the overall mean weight gain resulting from treatments varied from approximately one-half to two-thirds of a pound per animal per day.

The cost of spray material averaged one cent a day for each animal.

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A Twin-Rotor Broadcaster for the Application of Granulated Insecticides[1,2]

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ABSTRACT

Granular formulations of pesticides, due to ease of application, low residue hazard on food crops, and diminished hazard to applicators, are playing an increasingly important role in control programs for various insect pests. An applicator for the sheet distribution of granular materials has been tested, and its performance with various grades of attapulgite granules and granulated insecticides (under various types of field conditions) is described. This equipment has flexibility in that it may be adjusted to provide uniform deposits of the same material over various swath widths or to provide a range of swath widths with materials which vary greatly in particle density. This is accomplished primarily through the fact that delivery of the granules into the rotor blades may be adjusted over a 300-degree arc, making possible the selection of a location of the feed orifice for a given granulated material which will result in a uniform deposit on the ground.

Granulated insecticides may be useful in programs for the control of a variety of insects. For example, they have recently been used experimentally and routinely in mosquito control programs (Labrecque *et al.* 1956, Washburn 1956), and have suppressed insects infesting meadows and hay fields (Evans & Gyrisco 1955, Hansen & Dorsey 1957, Muka 1957). Broadcast application of various insecticides in granular formulations has been recommended for control of the imported fire ant (Hutchins & Green 1957). Corn rootworm populations have been lowered by the use of granulated insecticides (Lilly 1954), which have also been recommended for control of the southern corn rootworm (Hansen & Dorsey 1957). Other soil-inhabiting pests such as white grubs, symphylids, white-fringed beetles, and wireworms may also be controlled by the application of granulated insecticides. In addition to the recent increased use of granulated pesticides, there is a strong current tendency toward the granulation of fertilizers (Hill 1957). The development of suitable power-operated ground equipment for the soil surface application of granulated materials is therefore important for increased effective utilization of these formulations.

DESCRIPTION AND OPERATION OF EQUIPMENT.—In the course of investigations in the Coachella Valley on the control of *Hippelates* eye gnats, application of granulated insecticides on an experimental basis proved to be desirable. Numerous units of seeders and fertilizer applicators were investigated for the purpose of applying granu-

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[3] The authors acknowledge the assistance of Leonard D. Moore and Norman A. Lewis in this work.

FIG. 1.—Anderson twin-rotor broadcaster mounted on a jeep and employed in soil treatment with granulated insecticides for control of *Hippelates* eye gnats in a date garden.

lated insecticides to the surface of the soil. A new type of twin-rotor broadcaster[4] (fig. 1) proved to be effective for a sheet-type of application. Its chief advantage lies in the fact that it may be adjusted to provide uniform deposits of the same material over various swath widths or to provide a range of swath widths with materials which vary greatly in particle density. The performance of this equipment was tested with various grades of granulated materials.

Several modifications were made in the original design of the applicator. The shape and size of the feed orifices were altered to provide for more precise calibration in delivery of insecticidal granules. A suitable screen was installed on the top of the hopper to remove aggregates and prevent clogging and irregular feeding. A bushing placed over the shaft that turns the rotors decreased the leakage of materials from the hopper. Deflectors were installed to prevent forward throw of granules toward the operator. These changes, suggested to the manufacturer, are currently incorporated in the manufacture of commercially available units. It is believed that the efficiency of the applicator has been increased for the distribution of granulated insecticides.

The broadcaster used in these trials is powered with a 3- to 4-horsepower gas engine and mounted on a jeep (fig. 1). Two 4-blade rotors disperse the granules. By varying rotor speed, swath width may be varied by 15 to 30% (tables 1 and 2). This equipment has been most useful in soil applications in vineyards, citrus groves, and date gardens, where different planting distances call for varied single swaths or multiple swaths of the total width. At the higher speed levels of the rotors, however, increase in speed does not yield corresponding increase in swath width.

A large hopper with a capacity of approximately 400 pounds of attapulgite granules is mounted above the rotors. An agitator in the hopper, possibly of some use in seeding operations, is not needed for the delivery of free-flowing granulated materials. Two feed slots open at the bottom of the hopper, one above each rotor, and through these the granulated materials are fed by gravity. The equipment may readily be calibrated to provide consistent dosages.

By a combination of lever control and reversal of the removable orifice plates, the position of the feed aperture with respect to the rotor blades can be varied over an arc of approximately 300 degrees. This feature provides flexibility in dealing with materials having different particle weights. Flexibility in the position of the feeding aperture provides control over the general direction of discharge of particles from the rotors. There is only one setting of the feed aperatures for a given material that results in a uniform deposit. Swath width may be varied, however, by varying rotor speed, as previously noted. To achieve uniform broadcasting of heavier particles the feeding aperture lever is retarded; for lighter particles the lever is advanced. Retardation of the lever decreases the interval between the time of deposit of the material on the rotors and the time when the granules are thrown out into the air stream. Advance of the lever has the opposite effect.

A deflector forming an angle of approximately 25 to 30 degrees with the front vertical plane of the broadcaster keeps the granulated materials from flying into the front of the vehicle and becoming a source of discomfort and a hazard to the operator. Presence of a deflector, however,

[4]Anderson Farm Equipment Company, Los Banos, California.

Table 1.—Performance of the Anderson twin-rotor broadcaster (without deflectors) using aldrin formulations on various meshes of granulated materials.[a]

Material and Per Cent Concentration	Type of Granules	Mesh Size	Rotor Speed (r.p.m.)	Average Swath Width (Ft.)
Aldrin, 10	Attapulgite RVM-A	20/30	1000	25.0
			1200	29.5
			1500	32.0
			1750	32.0
Aldrin, 10	Attapulgite LVM-A	30/60	1000	17.0
			1200	20.0
			1500	21.0
			1800	21.5
Aldrin, 10	Attapulgite LVM-A	30/40	1000	16.0
			1200	18.5
			1500	20.0
			1800	19.5
Aldrin, 20	Attapulgite LVM-A	30/40	1000	17.5
			1200	19.0
			1500	19.5
			1800	21.5

[a] Three to six runs were made for each trial and the average of these values is indicated in the table. Swath widths measured on a background of black cloth.

Table 2.—Average swath widths obtained with the Anderson twin-rotor broadcaster, using deflectors on the sides and extending the upper and lower plates to the deflectors.[a]

Material and Per Cent Concentration	Type of Granules	Mesh Size	Rotor Speed (r.p.m.)	Average Swath Width (Ft.)
Aldrin, 10	Attapulgite LVM-A	30/60	1000	16
			1200	18
			1500	19
Aldrin, 10	Attapulgite LVM-A	30/40	1000	18
			1200	20
			1500	22
—	Attapulgite LVM-A	30/40	1000	13
			1200	15
			1700	15
—	Attapulgite RVM-A	30/60	1000	13
			1200	15
			1500	15
—	Attapulgite RVM-A	20/30	1000	18
			1200	20
			1500	22
—	Attapulgite RVM-A	15/30	1000	21
			1200	22
			1500	25

[a] Three to 10 runs were made for each trial. The figures for the measured swath width were almost the same for a given material and speed.

decreases the swath width (tables 1 and 2) by approximately 5 to 10%.

RESULTS AND DISCUSSION.—As would be expected, coarser meshes of attapulgite granules or attapulgite granular formulations resulted in wider swath widths than finer meshes. Impregnation of insecticide on the granules increased the swath width by as much as 30 to 45% (table 2). Increase in swath width of about 3 to 10% was observed when a 20% aldrin formulation was compared with a 10% aldrin formulation on 30/40-mesh-size attapulgite granules.

In order to determine the deposit pattern of granulated materials over the entire width of the swath, deposit catches were obtained at the center of the swath and at various distances from the center. Various meshes and types of attapulgite granules as well as 10% aldrin formulations were utilized in obtaining this information.

The deposit catches in lids from three ½-gallon ice-cream cartons, were composited and weighed. The results from these runs (table 3) indicated that this equipment yields rather uniform deposits. However, it is apparent from the data that the deposit pattern is slightly less uniform for lighter materials than for heavier materials, the deposits being slightly heavier at the center for the lighter particle materials. Deposits obtained with the heavier particle materials (e.g., 20/30-mesh granules) do not show this effect at the center of the swath (table 3).

Speed of the jeep on which the broadcaster was mounted, when varied over a range of 2 to 7.5 miles per hour, did not appreciably influence the swath width. Also, at rates varying between 15 and 100 pounds per acre, feeding rates did not result in appreciable change in the swath width at a given speed of the rotors.

As little as 10 pounds per acre of attapulgite granules can be metered through the feeding mechanism without

Table 3.—Swath widths and deposit patterns given by Anderson twin-rotor broadcaster using various meshes of attapulgite granules and aldrin granulated formulations.

Concentration	Mesh Size and Type of Granules	Swath Width (Ft.)	Combined Deposit Weights (in Mg.) at Center and at Various Distances (in Ft.) from the Center[a]											
			Left							Right				
			13	11	9	7	5	3	Center	3	5	7	9	11	13
Aldrin, 10%	30/60 LVM-A	17	—	75	190	550	555	400	625	410	525	525	190	100	—
Aldrin, 10%	30/40 LVM-A	19	80	150	450	420	350	300	450	350	500	500	250	90	50
—	20/30 RVM-AA	27	190	310	375	350	350	350	350	325	325	325	250	200	155
—	30/60 RVM-AA	17	—	100	200	390	535	460	600	450	520	350	120	80	—
—	30/40 LVM-A	16	—	50	100	425	560	400	500	450	555	375	100	50	—
—	30/40 LVM-A[b]	16	—	—	110	550	720	620	620	620	820	550	120	—	—

[a] Lids of three ½-gallon ice-cream cartons were set in a row at center and at each distance from the center. Deposit catches from all three lids were combined and weighed to the nearest 5 mg.

[b] This run had a larger hopper opening. Openings were the same for all the other runs.

clogging. However, at this rate of application the vehicle on which the broadcaster is mounted must travel at a speed of 7 miles or more per hour. As much as 250 to 300 pounds per acre of attapulgite granules can be applied by suitable adjustment of the standard feed orifice and vehicle speed.

The broadcaster has been tested extensively under field conditions in the experimental larviciding program for *Hippelates* eye gnats. Applications of granulated insecticides made with this equipment under a variety of conditions, such as those existing in date gardens, vineyards, citrus groves, and row crops, have resulted in uniform deposit patterns and desired dosages of toxicants.

The applicator seems to be suited for satisfactory service wherever a broadcast type of application is desired for distribution of a wide range of granulated materials.

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Biology and Control of the Purple-backed Cabbageworm in Newfoundland[1]

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ABSTRACT

The purple-backed cabbageworm (*Evergestis pallidata* Hufnagel) was first reported in Newfoundland in 1943 and is now considered a major pest of cruciferous crops throughout the province. Only one generation was observed. The insect passes the winter in the prepupal stage in an earthen cocoon at or near the surface of the soil. It pupates in June and the moths emerge during the latter part of July. Eggs are laid in masses on the undersurface of the lower leaves of susceptible plants, and hatch in 4 to 8 days. There are four larval instars.

The species was found to be unusually free from natural enemies, not a single parasite being reared from field-collected larvae in a 3-year study. Satisfactory control was obtained with DDT either as a spray or as a dust. A spray containing 1 lb. of 50% DDT wettable powder in 50 gal. of water applied to cabbage and turnips at 125 gal. per acre gave 90% reduction; 3% dust at 25 to 35 lb. per acre gave 83%.

The purple-backed cabbageworm, *Evergestis pallidata* Hufnagel, is known to have existed in America for over 100 years. It was first reported in the United States near Boston, Mass., by Harris (Scudder 1869, p. 322), and in Canada from Cape Breton Island by Fletcher (1903). It was first observed in Newfoundland in 1943 and is now considered a major pest of cruciferous crops throughout the province. Although *straminalis* Hbn. has been the name in common use for this species, *pallidata* Hufn. apparently has priority (Beirne 1952, p. 135) and is used in this paper. A native of the Old World, the species is widely distributed in central Europe and is common in parts of the British Isles (Beirne 1952). It is now found in 14 states of the United States and in nine provinces and one territory of Canada (fig. 1).

ECONOMIC IMPORTANCE AND HOST PLANTS.—In Newfoundland the species may be very numerous in one year and very scarce the next. When numerous it has been known to defoliate rutabagas and to tunnel into the roots themselves, thereby rendering them unfit for market. In cabbage, the larvae tunnel from the undersurfaces of the leaves up into the head, and, if numerous, completely hollow it out. In addition, the caterpillars are very unsightly and leave a large amount of excrement throughout the edible portion of the cabbage. Chittenden (1926) reported that in a serious outbreak on turnips near Pittsburgh, Pa., the larvae had eaten the leaves and then riddled the turnips. All cruciferous vegetables are attacked by this insect: turnips, cabbage, cauliflower, Brussels sprouts, kale, kohlrabi, and horse radish, the last being the most susceptible. When early rutabagas are grown along-side cabbage or late rutabagas, the early rutabagas are usually the more heavily infested. A few egg masses were found on shepherds purse, *Capsella bursapastoris* (L.) Medic. and on sheep sorrel, *Rumex acetosella* L., but the larvae were not found on these plants.

LIFE HISTORY AND HABITS.—Chittenden (1921) reported two generations of this species per year in Virginia whereas in Newfoundland there is only one. The female moth deposits her eggs in compact masses on the undersurfaces of the lower leaves of susceptible plants. The number of eggs per mass varies considerably, 56

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[2] Associate Entomologist.

FIG. 1.—Distribution of the purple-backed cabbageworm in Canada and the United States.

masses examined from 1950 to 1953 having 2 to 52 eggs. The egg is irregular oval in outline, flattened dorsoventrally with a translucent reticulate margin, and somewhat resembles a fish scale in appearance. On the average, including the margin, it is 1.1 mm. long, 0.8 mm. wide and 0.4 mm. thick. In the mass the eggs overlap one another for approximately half their length. The mass is a brilliant yellow with a waxy coating that makes it very conspicuous. As the eggs (fig. 5) become older they progressively change to brown and finally black. The black color just before hatching is due to the blackness of the head capsule of the developing larva within the transparent chorion. The eggs hatch within 4 to 8 days after oviposition.

When newly hatched, the first-instar larva is a pale watery green, with a medium-brown prothoracic shield. It is 1.5 to 2.0 mm. long. The head capsule ranges in width from 0.26 to 0.34 mm. with an average of 0.31 mm. Many minute dark-brown tubercles bearing one to several long tactile setae are present on the body. In this stage, the larva feeds exclusively on the undersurface of the leaf.

Table 1.—Duration of various stages of the purple-backed cabbageworm in the insectary at St. John's, Newfoundland, 1954.

Stage	No. Studied	Duration (Days)	
		Mean	Range
Egg	20 (masses)	7.2	4– 12
First-instar larva	42	9.1	4– 14
Second-instar larva	33	8.9	5– 12
Third-instar larva	21	10.3	7– 14
Fourth-instar larva	28	25.7	17– 43
Prepupa	29	263.0	256–272
Pupa	29	28.3	21– 35
Adult	68	13.4	8– 21

The second-instar larva is approximately 4 to 5 mm. long. The head capsule ranges in width from 0.46 to 0.62 mm. with an average of 0.55 mm. The head is black and the abdomen dark green dorsally and a pale watery green ventrally. The chalazae that bear the setae are very prominent with dark-brown, lightly chitinized centers. After the first ecdysis the larva becomes very active and eats holes through the leaves, causing very noticeable injury.

The third-instar larva is approximately 8 to 10 mm. long at first but grows considerably longer. The width of the head capsule varies from 0.84 mm. to 1.10 mm. with an average of 0.96 mm. The larva is dark olive-green dorsally, the ventral surface being lighter and having only a faint greenish tinge. Laterally a spiracular stripe of bright yellow runs the entire length of the body.

The fourth-instar larva is approximately 15 mm. long at first, but when fully grown is from 20 to 22 mm. The head capsule ranges in width from 1.44 mm. to 1.70 mm. with an average of 1.60 mm. The larva (fig. 6) is very robust and bristly. The dorsal surface is purplish-brown, the ventral somewhat ash-gray. A very conspicuous, narrow lateral yellow band runs the entire length of the body, with a very narrow white band along its ventral margin. The larva has four pairs of abdominal and one pair of anal prolegs. When fully grown it is nocturnal in its habits and usually hides between the leaves during the day. After feeding for 2 to 3 weeks it becomes sluggish, contracts, and soon leaves the host plant to spin its cocoon in the soil just below the surface. The larva has then contracted to about two-thirds of its original length. The transition to the prepupa occurs during the last week of September or the first 2 weeks of October. The winter is passed in the prepupal stage within the cocoon, where it remains until June of the following year, when it pupates.

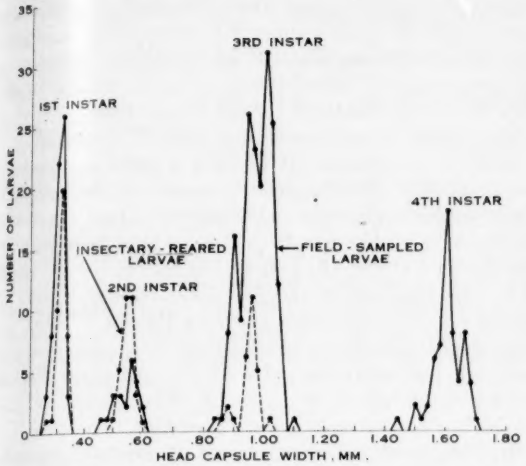
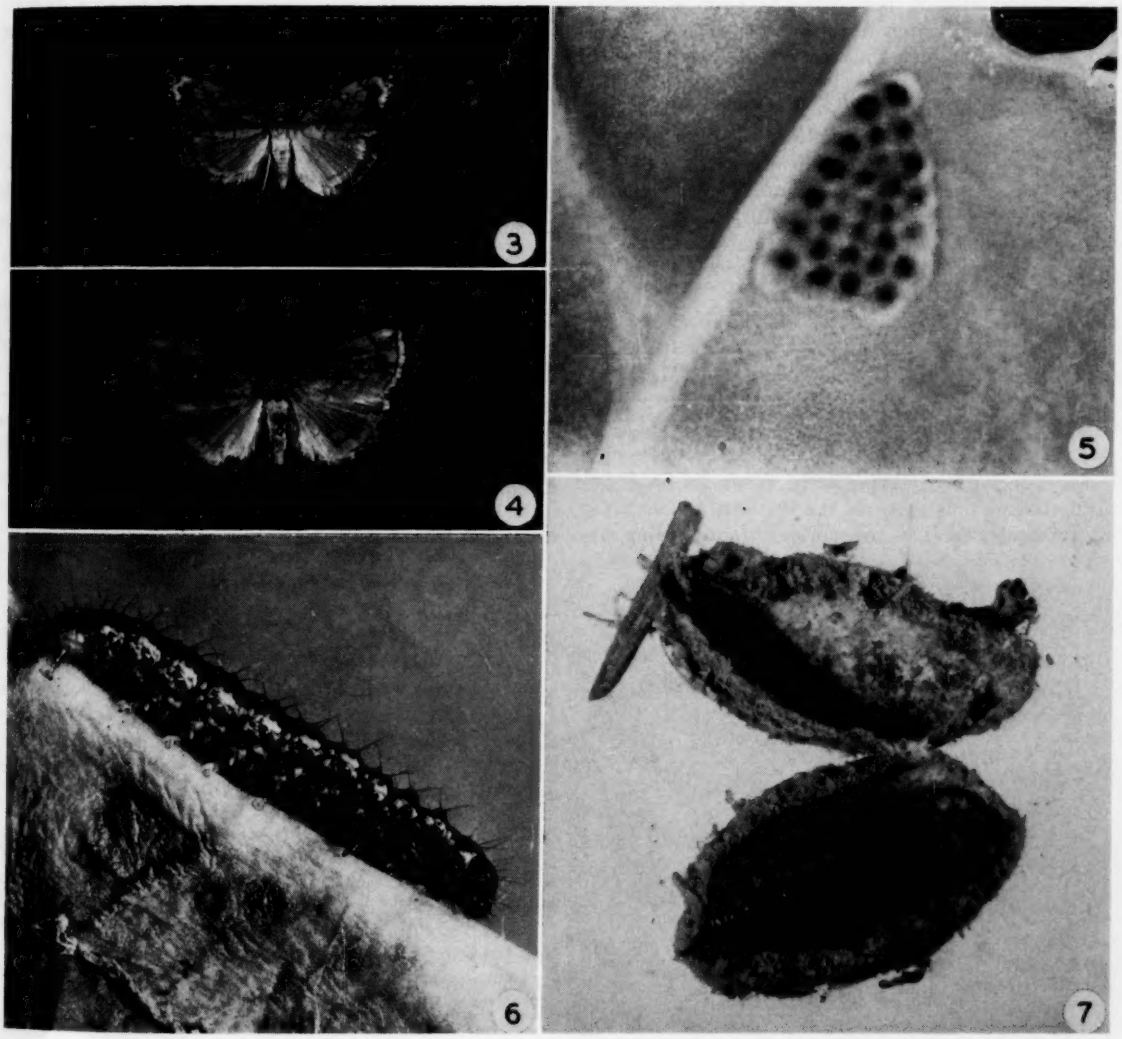


FIG. 2.—Frequency distributions of head capsule widths of insectary-reared and field-collected larvae, 1954.

Data on the number of larval instars and the duration of each (table 1) were obtained by rearing larvae in individual dishes in an insectary. These larvae were reared from egg masses collected in the field during early August, 1954. Daily observations were made during the entire period of development and strips of cabbage leaves were supplied for food as needed. After each ecdysis the cast-off head capsule was retained and its width measured with a microscopic micrometer. In addition, measurements of head capsules were made on larvae gathered in the field throughout the season. A total of 324 head capsules from field-collected larvas and 99 from insectary-reared ones were measured. The frequency distributions of head capsule widths of insectary-reared and field-collected larvae are shown in figure 2.

The cocoon (fig. 7), which is oval in outline, is 12 to 15 mm. long and 5 to 7 mm. wide. The interior of the cocoon is smooth and lined with dark gray silk. Exteriorly it is completely covered with soil particles evidently attached by a very viscid substance. Since the cocoons blend with the soil they are very hard to find.



FIGS. 3–7.—*Evergestis pallidata* Hüfn. 3, Male moth. 4, Female moth. 5, Egg mass before hatching. 6, Fourth-instar larva. 7, An open cocoon showing the contracted overwintering larvae in the prepupal stage.

Observations on development within 29 cocoons during the spring of 1954 showed that the larva pupates during the period from June 15 to 29. However, opening the cocoons may have influenced pupation. The pupa is light brown during its early development, but gradually changes to dark brown. The moth escapes from the cocoon through the loosely constructed end.

The moth is a medium-sized pyralid with a spread of ¾ to 1¼ inches. In color, it is straw yellow with irregular dark-brown lines. The males and females are similar in form and color (figs. 3 and 4).

The adult emerges during the latter part of July, duration of the pupal period being related to soil temperature. In 1952 the moths in the field emerged from July 23 to August 4; in 1953, from July 16 to August 3. In 1954, adults emerged in an insectary from July 18 to August 3, from 100 fourth-instar larvae that were placed on cabbage in each of two cages on August 20 and 26, 1953; the peak of emergence occurred on July 19 and July 25, respectively.

Five years' field observations indicated that the beginning of the oviposition period varied from July 16 to July 25 and the end from August 11 to August 19. The duration of the oviposition period varied from 17 days in 1950 to 29 in 1953.

CONTROL.—This insect is usually free from natural enemies. In experiments from 1953 to 1955, 50 fully grown larvae collected from a field were individually reared in vials in an insectary. The majority of these larvae overwintered and emerged as adults during the following year. There was no evidence of parasitism and not a single parasite was obtained. Chittenden (1921) reported that a single specimen of *Bracon montrealensis* Morrison was reared from larvae of this species in a 2-year study in the vicinity of the District of Columbia. Muesebeck (1923) listed *Meteorus autographa* Mues. as a parasite.

Since *E. pallidata* hibernates in the prepupal stage in a loosely constructed cocoon, deep, thorough, and frequent cultivation during late fall and early spring are important cultural control practices. As the cocoons are most frequeutly found near the soil surface, fall plowing also helps to destroy the overwintering larvae by burying them more deeply. It is advisable not to plant early varieties of rutabagas adjoining fields of late-maturing varieties, as the early varieties are very susceptible to infestation and may act as an attractant for the female moth.

The species is easily controlled with DDT as a spray or a dust. In an experiment in 1951 a spray containing 1 pound of 50% DDT wettable powder in 50 gallons of water applied to turnips at 125 gallons per acre gave 90% reduction in numbers of the larvae. Three per cent DDT dust applied at 25 to 35 pounds per acre gave 83% reduction. The numbers of purple-backed cabbageworms per turnip plant 7 days after applying the DDT spray and dust were 0.3 and 0.5, respectively. The untreated control plots had an average of 2.9 larvae per plant. Best results were obtained by applying these sprays or dusts when the first egg masses were beginning to hatch.

In Newfoundland, where there is only one generation of this pest per year, one spraying or dusting, if properly timed, is sufficient. For cabbage crops that are within a few days of being harvested 1% derris dust, which is nontoxic to warm-blooded animals, should be used. When applied at 25 to 35 pounds per acre, this material gave 76% reduction in numbers of the larvae in 1951.

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Dieldrin for Control of Fuller Rose Beetle Attacking Potted Citrus Trees in a Greenhouse[1]

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The Fuller rose beetle (*Pantomerus godmani* (Crotch)) became a serious pest in a greenhouse at the U. S. Department of Agriculture Horticultural Field Station in Orlando, Fla., during the summer of 1957. The adults were feeding heavily on the foliage and the grubs on the roots of potted citrus trees. The beetles were distributed over the entire greenhouse, which was 50 by 33 feet, with nine plant benches along each side of a central walkway.

On August 14 a spray of 3 pounds of 25% dieldrin wettable powder in 3 gallons of water was applied through a device attached to the water hose that mixed 1 part of the solution in 30 parts of water. The foliage was given fair coverage by means of a circular, flat sprayhead, and the soil in the pots was drenched. The following day 2 dozen dead adult beetles were found. Checks of the greenhouse for the next several weeks yielded from 5 to 12 dead beetles each day.

An infestation of mealybugs, soft scales, and red spiders became a problem, and on November 8 a spray consisting of 15% parathion wettable powder at 2 pounds, 57% emulsifiable malathion at 1 quart, and 43.7% emulsifiable Trithion at 1 quart per 100 gallons of water was mixed and applied with a 3-gallon tank sprayer. Eighteen gallons was sufficient to give good coverage of the foliage. Dead and dying beetles could still be found occasionally at the time of the November spray and for a few weeks afterward. No beetles have been found for over 4 months.

No phytotoxic effects have been observed as a result of the sprays.

[1] Accepted for publication March 31, 1958.

Laboratory Determination of Organophosphorous Insecticide Resistance in Three Species of *Tetranychus* on Cotton[1]

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Abstract

Two species of spider mites, *Tetranychus atlanticus* McG. and *T. pacificus* McG., suspected of resistance to treatment with organophosphorous compounds were collected from field cotton and reared in the laboratory. Subsequent laboratory evaluation indicated that both species were resistant to parathion and somewhat less resistant to Trithion. Neither species showed marked resistance to Kelthane or Aramite. A third species, *T. cinnabarinus* (Boisduval), in preliminary laboratory tests also showed resistance to parathion.

Since first suggested (Neiswander & Morris 1940), the phenomenon of mite resistance has been well documented in both the green house (Smith & Fulton 1951, Pritchard 1953, and others) and the field (Newcomer & Dean 1953, Cutright 1955, 1956, and others). Among the mite species listed as being resistant are *Tetranychus telarius* (L.) in the greenhouse (Smith & Fulton 1951, Smith 1952, Jefferson & Morishita 1956); *T. pacificus* McG. and *T. mcdanieli* McG. on deciduous trees (Newcomer & Dean 1953); *Panonychus ulmi* (Koch) on deciduous trees (Newcomer & Dean 1952, 1953, Cutright 1955); *Paratetranychus pilosus* (C. and F.) on deciduous trees (Lienk *et al.* 1952); and *Panonychus citri* (McG.) on citrus (L. R. Jeppson, unpublished data).

Early in the summer of 1956 the failure of demeton (Systox) to control *Tetranychus altanticus* McG. on cotton was reported from the Palo Verde Valley, California. Almost simultaneously similar failures were reported with *T. pacificus* on cotton on the west side of the San Joaquin Valley near Tranquility, California. Mite collections were made in both areas to determine the levels of resistance and evaluate responses to treatments with parathion, Trithion, Kelthane, and Aramite. For comparison, susceptible strains of each species were collected from fields in which no control difficulty had been experienced.

In response to reported control failures in 1957 a sample of *Tetranychus cinnabarinus* (Boisduval) was collected from cotton in the Coachella Valley and tested with parathion.

Materials and Methods.—Resistant and susceptible colonies of each species were established in the laboratory on Henderson dwarf lima bean plants. The plants were grown in perforated aluminum pans (9″×9″×2″) containing a coarse grade of vermiculite, and were suitable for use 8 to 10 days from the time of planting. The plants were then 4 to 6 inches high and both primary leaves were well developed. A single application of 1½ pints of Hoagland's nutrient solution was made to the trays in addition to the normal watering. Each mite colony was maintained in a separate redwood sand box (3′×3′×6″). Three sand boxes were located about 4 inches apart in a metal trough (11′×3′2″×6″) containing several inches of water. Besides providing moisture for the bean plants, the water formed a barrier which reduced the chances of cross-contamination between colonies.[2] The mite-infested bean plants were held in the center of the sand area and were enclosed by a rectangular cage (32″×28″×24″) covered with an inert vinyl plastic (fig. 1). A door at one side of the cage permitted access to the colonies. Fresh bean plants were added at the first sign of plant breakdown. Mites were transferred from one pan to another by removing the old mite-infested leaves and placing them on the new plants. As a safeguard against contamination, all personnel were required to wash their hands and arms prior to handling any mite colony.

The treatments were made by immersing mite-infested leaves in a known concentration of emulsified toxicant. In preparation for this treatment, individual bean plants were transplanted from the rearing trays into small bottles of nutrient solution. The plants were then infested by pinning mite-infested leaves from the appropriate mite culture onto the young plants. Ten to fourteen hours were allowed for the mites to transfer to the new plants. The leaves which had been pinned on were then removed. Ideally, 75 to 100 adult female mites would transfer to each leaf in this manner. Mites of all stages would also transfer to the young plants, but for the purpose of these tests only adult females were considered. These newly infested leaves were gently immersed in the test solution for 4 seconds and allowed to dry. A card (5″×8″) bordered with lanolin was placed under each plant to collect any mites dropping from the foliage.

Concentrations of the test materials were prepared from a 25% solution of the technical material in Xylene. An emulsifier[3] was added at the rate of one drop (2.8 mg.)

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[2] The equipment and rearing methods described are modifications of a technique developed by R. N. Jefferson and F. S. Morishita, Department of Entomology, University of California at Los Angeles.

[3] Triton X-100 (alkylated aryl poly-ether), manufactured by Rohm & Haas Co. Philadelphia, Pa.

Fig. 1.—Plastic-covered cages and other equipment used in rearing colonies of *Tetranychus* mites on lima bean plants.

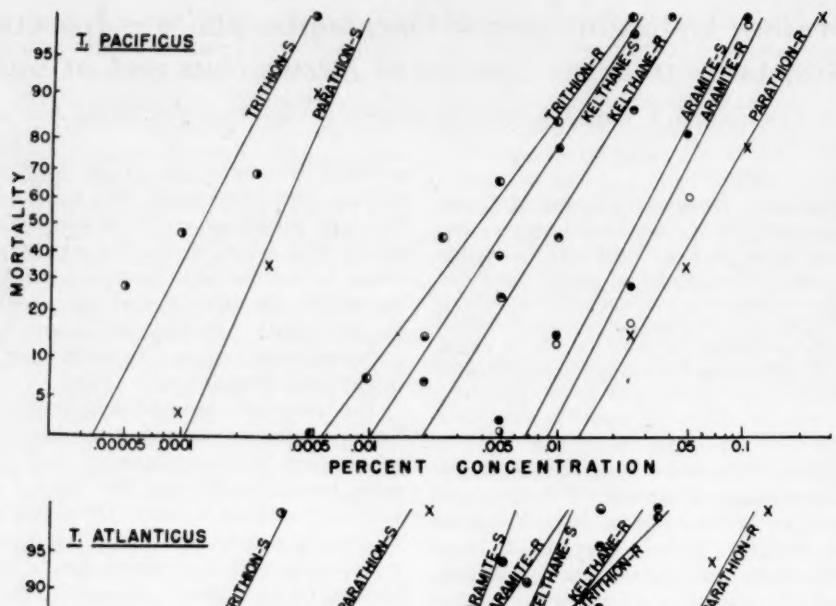

FIG. 2.—Dosage-mortality curves of resistant (R) and susceptible (S) strains of *Tetranychus pacificus* and *T. atlanticus* in response to Trithion, parathion, Kelthane, and Aramite.

per 25 ml. of solution. Mortality counts of adult female mites were made 24 hours after the treatment. The per cent mortality was based on the total number of dead and live adult female mites present on the card and on both bean leaves. Each plant was considered one replicate. All dosages were replicated only once on any 1 day, but were repeated on three different days. The dosage-mortality curves in figure 2 are based on the average of these treatments. The LC-50 values for resistant and susceptible strains of the two species in response to the four materials tested are shown in table 1 for comparison.

RESULTS.—From the mortality curves (fig. 2) it is apparent that resistance to parathion and Trithion is present in both *Tetranychus atlanticus* and *T. pacificus*. The level of resistance to Trithion, however, is lower than that to parathion. The reduced slope of the mortality curve of the resistant strain of *T. atlanticus* in response to Trithion may indicate considerable variation in the factors governing resistance to this chemical. The response of the susceptible and resistant strains to Kelthane and Aramite suggests that there is no resistance to these materials. However, the Aramite and Kelthane dosage-mortality curves of the resistant strain of *T. pacificus* both show a slightly higher LC-50 than those of the susceptible strain. These slightly higher LC-50 levels may be correlated with resistance to organophosphorous compounds.

A single comparison of the resistant *Tetranychus cinnabarinus* against a susceptible strain indicates resistance to organophosphorous compounds. The degree of resistance is similar to that in the other species, although further tests will be required to establish the exact level.

DISCUSSION.—The development of resistance by *Tetranychus atlanticus* and *T. pacificus* to organophos-

Table 1.—LC-50 values for resistant (R) and susceptible (S) strains of *Tetranychus atlanticus* and *T. pacificus*, based on the percentage concentrations shown in figure 2.

	T. pacificus		*T. atlanticus*	
MATERIAL	Resistant	Susceptible	Resistant	Susceptible
Parathion	0.06	0.0003	0.045	0.0005
Trithion	.0035	.0001	.005	.00015
Aramite	.03	.027	.003	.0035
Kelthane	.009	.006	.007	.006

phorous compounds is the culmination of a selection in which the most resistant individuals have been brought together to form a genetically fixed resistant strain. The selective agents have been various phosphorous pesticides. The stability of these resistant strains apparently depends on the genetic variability of the mites and may vary in subsequent tests with phosphorous-containing poisons. Reversion of resistant strains to susceptibility has been reported by Garman (1950) in the laboratory and by Cutright (1956) in the field, although according to Smith & Fulton (1951) in laboratory tests this did not occur.

The selection of resistant individuals and the rate of development of a genetic strain depend, at least in part, on the severity of the selecting agent, in these cases repeated exposures to organophosphorous pesticides. In studying the effect of this factor on the appearance of resistance in the field, certain considerations should be made in the case of polyphagous organisms of this type. Both mite species will attack a number of wild and cultivated host plants including cotton and alfalfa, the latter being one of the most widely grown crops in California. Thus an evaluation of the number of exposures to organophosphorous poisons should include not only the treatments applied to the hosts on which resistance first appears but on all other nearby hosts as well. The use of organophosphorous compounds for the control of other pests on these same hosts should also be considered. In spite of the movement of portions of the population by means of air drift, a reservoir of mites remains in each field more or less subject to the treatments given that field. Thus any practice which increases the number of organophosphorous applications in turn increases the selection rate of resistant strains.

The early-season failure of demeton to control *Tetranychus atlanticus* on cotton in the Palo Verde Valley suggests that resistance developed on nearby alfalfa fields, probably in response to the frequent organophosphorous treatments for the spotted alfalfa aphid, *Therioaphis maculata* (Buckton). The mites from the alfalfa later migrated onto the newly emerged cotton plants. In the San Joaquin Valley the appearance of resistance in *T. pacificus*, which developed prior to the appearance of the spotted alfalfa aphid, was also very likely the result of an unusual frequency of treatments for pest control.

The method by which mite resistance can develop indicates that resistance may appear in any area in which acaricides or nonselective insecticides with acaricidal activity are applied frequently. In addition, resistance may spread from areas in which it has already developed. A treatment program with a positive means of preventing the development of resistance is unknown at this time; however, the establishment of sound pest control practices with the intent of reducing the number of treatments or exposures is of prime importance. In those areas in which resistance to organophosphorous compounds is already a problem, the use of chemically nonrelated materials such as those tested have proved satisfactory thus far.

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Aphids Provide Substitute Sugar Source for Honey Bees Following Freeze[1]

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Most entomologists have observed different species of insects visiting aphids in order to feed on the honeydew which they secrete. For example, shortly after cucurbits become infested by aphids one might observe ants, bees, wasps and even flies visiting the colonies.

On March 27, 1955, after several weeks of warm spring weather, there occurred a hard freeze (19° F.) which killed all of the many flowers at Florence, S. C. During the following week, temperatures returned to normal.

Certain varieties in the small grain variety yield test at the Pee Dee Experiment Station at Florence had been heavily infested with aphids prior to the freeze. Examination on April 1 showed that these aphids were apparently unaffected by the freeze and were, in fact, serving as a source of food to great numbers of honey bees. With all flowers in the area killed, the bees were feeding on the sweet, sticky exudation of the aphids.

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Laboratory Rearing of the Boll Weevil: A Satisfactory Larval Diet and Oviposition Studies[1]

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ABSTRACT

Five generations of the boll weevil, *Anthonomus grandis* Boh., were reared aseptically from egg to adult on a semisynthetic diet containing soybean protein, sucrose, corn oil, cholesterol, choline, vitamins, yeast extract, salts, cellulose, alginate, agar, and water. The adults were allowed to feed and oviposit on squares. Average egg production was three eggs per female per day, with a maximum of seven eggs. Cotton cotyledons also were found to promote oviposition of newly emerged adults. Weevils fed but did not oviposit on artificial diet unless cotton plant extracts were added. Oviposition also occurred on squares without bracts and squares that had been ground, remolded, and coated with paraffin.

The biological characteristics and the insecticide responses of the boll weevil, *Anthonomous grandis* Boh., have been studied a number of years under various conditions in the field and in the insectary. The natural characteristics have shown a wide range, so that it has been difficult to interpret experimental results in terms of standard behavior. Another factor that hinders research on this insect is its scarcity throughout the year. Only during a part of the cotton-growing season are boll weevils readily available. Therefore, an artificial rearing method would be desirable for a continuous supply of nearly uniform weevils for experimental purposes.

This paper reports a method for rearing this insect aseptically from egg to adult on semisynthetic diets. Studies on the oviposition of field-collected and laboratory-reared weevils on various diets also are discussed.

LABORATORY REARING.—Diets with casein and amino acids were prepared as described for the pink bollworm (Vanderzant & Reiser 1956b, Vanderzant 1957). A diet containing a modified soybean protein instead of casein was adopted as the standard larval diet. The composition of this diet is as follows:

CONSTITUENT	GRAMS PER 100 GRAMS OF DIET
Soybean protein (Drackett assay C-1)	5.0
L-Cysteine·HCl	0.05
Glycine	0.1
Sucrose	5.0
Corn oil (Mazola)	0.5
Cholesterol	0.05
Salts, Wesson's (Nutritional Biochemicals Co.)	1.0
Choline chloride (in 1 ml. water)	0.1
Cellulose, powdered	4.0
Sodium alginate (Kelcosol)	0.5
Agar (Difco Bacto granules)	3.0
Bacto yeast extract (Difco)	1.0
Vitamin mixture, 1 ml.[3] (Vanderzant 1957)	
Water to 100 grams	

The constituents were thoroughly mixed in a Waring blendor. In the final step the pH was adjusted to 6.5 with 2*M* potassium hydroxide. The diet was heated in a boiling-water bath until the agar dissolved, and then allowed to cool and harden. Pieces were cut and put in 17×60 mm. rearing vials. The mixture also could be dispensed while hot from a polyethylene squeeze bottle. The vials were plugged with cotton and sterilized at 15 pounds' pressure for 20 minutes. When the diet was cool, a hole for the egg was cut in the diet in each vial. Large numbers of vials may be filled, stored in a freezer, and autoclaved as needed.

Eggs were obtained from cotton squares. The bracts and the outer layers of the buds were cut away with a dissecting knife and the exposed eggs removed. Initially, squares collected from the field were used, but the yield of unhatched eggs was low. Subsequently, weevils were placed in cages and supplied daily with fresh squares in dishes of wet sand for feeding and oviposition. With an adequate supply of squares for food, as many as 30 eggs were deposited in a single square. It was thus possible to obtain about 100 eggs per hour. One medium-sized square was provided for every three newly emerged weevils, but after they had fed 10 days one square for every six weevils was sufficient.

The eggs were washed for approximately 5 minutes in a beaker of sterilizing solution of the following composition: 0.25 gram of mercuric chloride, 6.5 grams of sodium chloride, 0.5 gram of sodium glycocholate, 1.25 ml. of concentrated hydrochloric acid, 250 ml. of ethyl alcohol, and 750 ml. of distilled water. They then were rinsed once in a petri dish containing sterile water. Egg-washing apparatus previously described (Vanderzant & Reiser 1956a) could be used. Eggs were transferred aseptically with sable brushes to the rearing vials. Each egg was placed in or near the hole so that upon hatching the legless larva could work its way into the hole and was confined so that it could feed by pressing against the diet. The rearing vials were placed in an incubator at 86° ± 1° F. and a relative humidity of 80 to 90%. They remained under these conditions except for periodic observations until adults emerged. Adults then were removed, weighed, and placed for feeding and oviposition in 6×6×6 inch screened cages, not more than 30 weevils to a cage.

Eggs hatched in 2 or 3 days after they were placed on the diet. The larvae moved toward the bottom of the vials, and the insects often were not seen again until the adults emerged. Larvae that worked their way to the surface of the diet starved to death unless they fell into the hole again. If more than one egg was placed in a hole in the diet, only one adult was reared. It therefore appears

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[2] In cooperation with the Texas Agricultural Experiment Station, College Station.

[3] Contains 1.0 mg. niacinamide, 1.0 mg. calcium pantothenate, 0.25 mg. thiamin hydrochloride, 0.50 mg. riboflavin, 0.25 mg. pyridoxin hydrochloride, 0.25 mg. folic acid, 0.02 mg. biotin, and 0.002 mg. vitamin B_{12}. Folic acid and biotin were dissolved with the aid of alkali.

that the larvae are cannibalistic when crowded. Eggs placed in two holes on opposite sides of a vial usually yielded two adults. The adaptation of this method to a larger container such as a petri dish should be practical if some simple method of distributing the eggs aseptically into holes punched in the diet could be devised. A few adults were reared in this manner in petri dishes on the standard larval diet (Davich 1957).

In the summer of 1956 several mixtures of nutrients were tried as rearing diets. Amino acid and casein diets that had given excellent results in rearing the pink bollworm were tried first. On these diets most larvae molted once and some lived as long as 30 days, but none reached the pupal stage. When 1 gram of yeast extract was added to 100 grams of the casein diet (Vanderzant & Reiser 1956b), 79% of the larvae became adults. Thirty-four adults had an average weight of 8.3 mg.

Simultaneously with the attempts to devise a diet for rearing the larvae, diets were prepared for adult oviposition. Several of these diets were tried as larval diets. One diet containing soybean protein instead of casein, and 1 gram of yeast extract per 100 grams, permitted 78% of the larvae to become adults, with an average weight of 10.0 mg. Eleven adults reared on a similar diet without yeast extract had an average weight of 6.9 mg. Unfortunately, the number of eggs used for these experiments was limited because of the difficulty of getting eggs from squares collected in the field. Several hundred squares often were required to furnish 30 to 50 eggs.

In March 1957 a laboratory colony of boll weevils was started from about 50 adults obtained from Mexico. On the basis of preliminary data the soybean-yeast extract diet was chosen as the standard with which the other diets again were compared. No growth was observed in the diet containing casein alone. The growth on the casein diet with yeast extract added was not as good as that obtained previously in 1956. Only 29% of the larvae became adults. Twenty-seven adults had an average weight of 5.3 mg.

The yeast extract used in 1957 was from a different batch than that used in 1956, which might be one factor responsible for the difference in the results. In 1957 growth on diets containing soybean protein with and without yeast extract was similar. In 1956 much better development was observed when yeast extract was included. The proteins used came from the same batches. The difference in the batches of yeast extract and the small number of weevils used in 1956 may account for the results.

In table 1 data are presented for five generations of the boll weevil reared on the standard diet. Adults were given squares as food and oviposition sites for the eggs of the next generation. Seventy-seven per cent of the eggs became adults, 6.3% of the vials were discarded because of contamination, and 16.6% of the insects died, mainly in the egg or larval stage. Approximately 9% of the eggs did not hatch. Injuries to the eggs undoubtedly occurred from handling and the possible toxicity of the sterilizing solution also may have impaired hatching. Almost all unwashed eggs appeared to be fertile.

The adults were weighed together to get average values, but were nearly uniform in size and compared favorably with square-reared weevils. Also there was little variation over the five generations. The period from egg to adult averaged 16 days, with a range of 12 to 20.

Table 1.—Boll weevil adults reared on the standard diet.

Generation	Number of Eggs	Number Dead[a]	Number of Contaminated Vials	Number of Adults	Average Weight (Milligrams)
1	819	139	27	653	9.0
2	356	52	38	266	8.9
3	327	37	35	255	8.4
4	435	85	27	323	8.6
5	211	43	8	160	8.7
Total or average	2148	356	135	1657	8.8

[a] Unhatched eggs, larvae, pupae, and adults.

OVIPOSITION OF FIELD-COLLECTED WEEVILS.—In the summer of 1956 attempts were made to induce weevils to oviposit on artificial diets in the laboratory. The diets were those used for the pink bollworm and the boll weevil as described above, and combinations of these with crude materials such as dried ground cotton leaves, mixed pollen, and cottonseed meal. Sodium carboxymethylcellulose (CMC), 5 to 7.5 grams per 100 grams, was added to all diets as a thickening agent. Pieces of diet then were autoclaved and dipped in melted paraffin to retard desiccation. No eggs were found in any of these diets, although weevils fed readily through the paraffin coating.

When fresh cotton squares were ground in a Waring blendor, mixed with CMC, reshaped into balls, and coated with paraffin, weevils oviposited in these remolded squares. In table 2 data are given for oviposition in squares, with and without bracts, and in remolded squares enclosed in bracts taped together at the top and without bracts. Eggs were found in each case. The appearance of eggs in the bractless squares eliminated thigmotropism, thought to be caused by the involucre, as a factor in inducing oviposition.

Ether and ethanol extracts of cotton squares, blooms, and leaves were obtained from the Division's Pesticide Chemicals Research Laboratory at Beltsville, Md. These extracts were incorporated into six portions of the standard boll weevil larval diet, CMC was added, and pieces of diet were dipped in paraffin as described previously. The results obtained with these modifications are

Table 2.—Oviposition by field-collected boll weevils in cotton squares.

Squares	Number of Squares	Days in Test	Number of Eggs
Original			
In bracts	9	1	5
	4	2	6
Without bracts	16	1	8
	4	2	3
Remolded			
In bracts	3	2	1
Without bracts	9	2	5

Table 3.—Oviposition by field-collected boll weevils in the standard larval diet containing extracts of cotton plant parts.

Plant Part	Extract	Number of Squares	Number of Eggs in 48 Hours
Blooms	Ether	12	2
	Ethanol	7	1
Squares	Ether	9	0
	Ethanol	8	1
Leaves	Ether	6	0
	Ethanol	6	0

presented in table 3. Feeding was medium to heavy, but relatively few eggs were obtained. No eggs were found after 24 hours. After 48 hours four eggs were obtained in diets containing the ether extract of blooms and the ethanol extracts of blooms and squares. These tests were conducted late in the season with weevils that had fed mainly on bolls and hence produced very few eggs.

This experiment was repeated with a control cage containing cotton squares. Only one egg was deposited in the squares by 40 weevils after 48 hours. With the diet containing the ether extract of squares only one egg was obtained in 24 hours. No eggs were found in the diets containing the other extracts. Two cages with 20 weevils and 8 pieces of diet per cage were used for each test.

It is now known (Brazzel 1957) that a large percentage of the boll weevils collected in the field in the late summer are already in diapause even though they may still be feeding on the cotton plant. A large number of weevils in diapause would account for the poor results obtained in the last tests.

Oviposition of Laboratory-Reared Adults.—*On Cotton Squares.*—Newly emerged adults were caged with cotton squares as previously described. The sex was determined after the weevils had been caged for several days, and approximately equal numbers of males and females were found in each cage. Initially the cages were kept in a dark incubator at a mean temperature of 86° F. and the weevils were exposed to light during the day only when the doors were open and the squares were being changed. Later an incandescent bulb was placed in the incubator and 12 hours of light were provided daily. No obvious difference in oviposition was noted under these two conditions.

The oviposition record of females reared on the standard larval diet is given in table 4. The first egg usually appeared after 3 days of feeding on squares. Subsequently, the number of eggs gradually increased and reached a maximum between 10 and 15 days. At the peak of oviposition an average of 7 eggs per female per day was obtained, although the average for a 30-day period was about 3 eggs per day. Natural differences in the egg-laying capacities of weevils probably account for the differences between groups. Unfortunately, a shortage of squares made it impossible to get 30-day oviposition records for all groups. Adult mortality was about 10% in a 30-day period. Females survived longer than males.

On Cotton Cotyledons.—Owing to the obvious advantage of growing seedlings rather than squares, studies were undertaken to determine the effect of feeding cotyledons upon oviposition. No reports on such feeding have appeared in the literature. Two cages, each with 10 newly-emerged weevils, were provided with 1-day-old seedlings, which were kept fresh in a dish of wet sand. The seedlings were changed daily and examined for eggs. On the fourth day eggs were found on the surface of the cotyledons. However, weevils preferred the unfolded cotyledons in which they could hide.

Table 4.—Oviposition of laboratory-reared boll weevils on cotton squares and cotyledons.

Generation	Number of Females	Number of Eggs: At 20 Days, Total	At 20 Days, Per Female[a]	At 30 Days, Total	At 30 Days, Per Female[a]	Number of Dead Females
		On Squares				
1	4	164	41	295	74	0
	10	444	44	766	78	1(27)[b]
2	10	602	67	871	102	2(15) 1(26)
	10	700	70	—		0
3	9	369	41	—		0
4	10	430	43	—		0
5	10	439	46	—		1(16)
		On Cotyledons				
1	6	80	13	—		0
	5	87	17	—		0

[a] Corrected for dead females.
[b] Figures in parentheses indicate day weevil died.

Loss of eggs no doubt occurred from eggs falling into the sand and from weevils eating the stems so that the cotyledons dropped off and wilted. A satisfactory means of preventing this loss was devised. A small funnel was fitted with two filter papers with washed cotyledons between them. The stem of the funnel was provided with a wick and placed in a jar of water to keep the cotyledons from drying. Weevils crawled between the filter papers, fed, and deposited their eggs on the cotyledons. The maximum number of eggs per female per day was 2, and the average about 1 egg.

In another test 10 females that had fed on squares for 2 weeks were given seedlings alone for 1 week. Oviposition decreased sharply at first, but rose again to the oviposition rate for squares, 5 eggs per female per day by the fifth day. Incomplete data indicated that egg production thereafter would decrease slowly if weevils were not given squares again. As many as 10 eggs were found on a single pair of cotyledons. When the two cotyledons were folded together, the eggs were usually placed between them through holes eaten in one cotyledon.

Weevils given a choice of squares and seedlings fed and oviposited on both, but preferred the squares for oviposition.

Apparently the cotyledons do not provide such concentrated food as the squares, and this undoubtedly accounts for the low production of eggs. Cotyledons should be valuable as a supplementary food and as a means of keeping weevils in a healthy egg-laying condition should a shortage of squares occur. Mortality of weevils fed cotyledons was about the same as for squares.

Fifteen weevils placed in a cage with mature cotton leaves were reluctant to feed on them. No eggs were laid and within 2 weeks all the weevils were dead.

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Wireworm Behavior in Response to Chemical Seed Treatments[1,2]

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ABSTRACT

Orientation reactions of the wireworm, *Melanotus communis* Gyll., to various insecticide seed treatments were studied by following the movements of individual larvae tagged with radioactive cobalt in the soil.

It was found that the repellency of certain insecticide seed treatments to wireworms apparently has two components: (1) inhibition of the feeding reaction, and (2) orientation of the insects away from treated seeds. For convenience these are referred to as type one and type two repellency, respectively. Type two repellency does not preclude contact with treated seeds, and varies in degree depending on the insecticide used.

Aldrin, dieldrin, endrin, heptachlor and lindane seed treatments all appeared to possess considerable type one repellency. Type two repellency was most pronounced with lindane-treated seeds, and with aldrin-treated seeds where this insecticide was not used in excess of 1 ounce of actual toxicant per bushel of seed. Dieldrin, endrin and heptachlor seed treatments showed a degree of type two repellency intermediate between lindane and aldrin on the one hand and untreated control seed on the other.

Treatment of seed with organic insecticides to control soil insects is an economical method of insect control. Powdered formulations of lindane and dieldrin are presently the most widely used materials for this purpose. These insecticides have been commonly recommended for treating seed corn, usually at rates giving about 1 ounce of actual toxicant per bushel of seed, with the reservation that they are primarily effective against seed corn maggot, seed corn beetles and possibly Collembola. It is quite generally accepted that these treatments help check low infestations of wireworms, but that they do not afford much protection against heavy populations (Lilly 1956).

Although lindane and BHC seed treatments have sometimes been reported effective against wireworms, several suggestions of a possible repellent action of these chemicals to wireworms have accompanied the favorable reports from their use (Greenwood 1947, Jameson *et al.* 1947, Dogger & Lilly 1949, Lange *et al.* 1949, Faber 1951). Lilly (1953) has suggested that such repellency may be partially responsible for the failure of seed treatments to protect young corn where wireworms are numerous.

This paper describes a radioactive tracer technique used to study the orientation responses of wireworms to treated and untreated seed corn in the laboratory, and presents the results of this study. Similar techniques for tagging wireworms with radioactive cobalt-60 have been reported by Arnason *et al.* (1950), Fuller *et al.* (1951) and Frederickson & Lilly (1955).

MATERIALS AND APPARATUS.—The wireworm, *Melanotus communis* Gyll., was collected in the spring and early summer from bait traps of spoiled shelled corn located in corn fields near Britt in Northern Iowa. These insects were brought to the laboratory and stored in moist soil in containers which were kept in a cool place until the larvae were used.

Radioactive cobalt-60 wire, obtained in pieces 1 millimeter long by 0.5 millimeter in diameter and gold plated to prevent corrosion, were used to tag the wireworms. The activity of these sources was about 60 microcuries each.

The radiation detector was a Gieger-Mueller tube (type TGC-8, manufactured by Tracerlab, Inc., 130 High St., Boston 10, Mass.) connected by means of a three-foot cable to an analytical count rate meter (Model 1620, manufactured by the Nuclear Instrument and Chemical Corp., 223 West Erie St., Chicago 10. Ill.).

A film badge was always worn while working near the radioactive sources to detect any appreciable absorption of radiation by the body of the investigator. Health-physics precautions were always observed, and the possibility of injury to the investigator from radiation was very small.

The insecticides used were wettable powders containing 75% of aldrin, dieldrin, heptachlor, and lindane, respectively, and 50% endrin.[5]

PROCEDURES.—Chemicals were applied to 0.8-pound lots of seed corn contained in quart Mason jars. The rate of application was calculated and is reported in terms of ounces of actual insecticide per bushel. Five milliliters of 2.5% methyl cellulose solution per jar were used with the wettable powder formulations to aid in sticking them to the seeds. This amount was added to each jar and distributed evenly over the seeds before the dry materials were added. Each of the jars was shaken by hand for a

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[5] Supplied by the Mackwin Company, Shell Chemical Corporation, Velsicol Corporation, California Spray-Chemical Corporation, and Shell Chemical Corporation, respectively.

FIG. 1.—Pyrex tray containing a wireworm which is being held with a notched and folded index card prior to attachment of the cobalt-60 tag (at tip of forceps).

few seconds before being placed on the motor-driven laboratory mixer described by Starks & Lilly (1955), for 5 to 10 minutes. All seed lots received 0.27 gram of 75% captan powder, which is equivalent to 0.5 ounce of captan per bushel. This fungicide was added with further mixing after the insecticides had been applied.

An index card was folded twice and notched so as to fit snugly over the posterior portion of the penultimate segment of a wireworm to aid in attaching the radioactive tag to the insect (fig. 1). While the larva was held firmly beneath this card in the bottom of a large Pyrex tray, the dorsal surface of the last segment was cleaned with a small camel's-hair brush dipped in acetone. The dorsal surface of this segment was next sprayed with an acrylic base plastic spray while the index card was held in a vertical position to protect the major portion of the insect from the spray. After a few seconds the cobalt tag was placed transversely across the caudal segment perpendicular to the long axis of the insect's body. After tagging, the larvae were kept separated in small boxes containing moist soil until the following day when a test was begun.

The tests were conducted in earthen crocks, 11 inches in inside diameter and 6 inches deep (fig. 2). These crocks contained sifted soil to a depth of 3 inches and experimental seeds planted approximately equidistant from the soil surface and the bottom of the crocks. The soil was a dark (Webster) clay loam in which moisture was maintained at about 25% (dry weight basis) by adding the necessary amount of water to each crock daily. Four seeds were always planted together in a small hole $\frac{1}{2}$ inch in diameter by $1\frac{3}{4}$ inches deep. One tagged wireworm was released head-down in each crock in a small hole in the soil made with the point of a pencil. This was done to insure entrance of the insect into the soil at the desired point of release.

Eight observations of each insect's position in each crock were made daily at 2-hour intervals for 6 or 8 days. A glass pane, marked with a grid of 1-inch squares and with alphabetical and numerical coordinates, was used to aid in locating and recording the position of the larva in the horizontal plane. This pane was placed over the crock (fig. 2) and the Geiger-Mueller tube was moved about on the glass surface until the position of maximum needle deflection on the count rate meter scale was found. The wireworms used were between $\frac{7}{8}$ and $1\frac{1}{2}$ inch long and carried the radioactive tags on their caudal segments. Since each crock contained only 3 inches of soil, and the second inch in the vertical plane was largely occupied by seed at that point or points in the horizontal plane where seeds were planted, it was considered unnecessary to determine the insect's position in the vertical plane.

At the termination of a test all larvae were taken from the crocks and the tags removed with a small pair of forceps. The larvae were placed in individual tin boxes with moist soil and wheat as a source of food. Each larva was observed and provisioned (with food and moisture as seemed necessary) at weekly intervals thereafter until its death or until these studies were terminated. After the larvae had been taken from the crocks the seedlings were removed and examined for signs of wireworm injury. The only injury observed was that resulting from wireworm feeding on the seeds.

Two experiments were carried out, each consisting of a

number of separate tests.

Experiment I.—This experiment was designed to study the orientation responses of wireworms to aldrin, dieldrin, endrin, heptachlor and lindane seed treatments when these insecticides were applied at rates equivalent to 0.5, 1, and 2 ounces of insecticide per bushel of seed.

The experiment was composed of five tests or replications. In each test observations were made on five different wireworms, one in each of five crocks. Sixteen seeds were planted in each crock: four at each of four horizontal positions, these four positions being located 90 degrees apart and near the periphery of each crock. Seeds treated with one of the five insecticides at each of the three different rates of application were planted in each crock, along with seed that had received no insecticide as a check. These four treatments (0.0, 0.5, 1.0 and 2.0 ounces of insecticide per bushel) were always randomly assigned to the four planting positions in each crock.

The seeds were planted and the proper amount of water added to each crock in the evening prior to the release of a tagged wireworm at the center of each crock on the following morning. A total of 64 observations were made on the location of the larva in each crock during 8 consecutive days before a test was terminated.

Experiment II.—This experiment was designed to study the orientation responses of wireworms to seed treatments with the same five insecticides applied at the "standard" rate of 1 ounce of actual toxicant per bushel of seed. In contrast to Experiment I, which might be considered a choice-chamber situation in most respects, Experiment II confined each larva to an environment containing only one seed treatment.

This experiment consisted of six separate tests or replications. Observations were made on six wireworms, one in each of six crocks, during each test. Four seeds treated alike were planted at the center of each crock prior to the release of a single tagged larva near the periphery of the crock on the following morning. In each test a control crock contained seeds which had received no insecticide. Eight observations of the wireworm's location in each crock were made at 2-hour intervals each day for 6 consecutive days before a test was terminated.

RESULTS.—*Experiment I.*—When the sums of the recorded (i.e., the minimum) number of inches traveled by each larva during the fifth, sixth and seventh days of the tests were averaged for all crocks containing the same insecticide treatments, the averages for the different insecticides were: endrin, 46; dieldrin, 43; lindane, 27; heptachlor, 24; and aldrin, 10.

Observations to determine the length of life of each wireworm following the completion of the tests revealed that some larvae were still alive but obviously abnormal when these studies had to be terminated. It is assumed, for purposes of comparison, that in these cases death was assured at the time of the last observations. The average lengths of life in weeks after the insects were exposed to the respective insecticide treatments were: aldrin, 10; heptachlor, 12; dieldrin, 14; endrin, 16; and lindane, 19.

The duration of the longest visit made by each larva to the vicinity of each seed treatment is found in table 1. These data indicate a tendency for larvae to remain for longer periods of time in the vicinity of seeds treated with 2 ounces of aldrin per bushel than with seeds treated with 0.5 or 1 ounce of this insecticide or with seeds receiving no insecticide treatment. This suggests that chance contact with a sufficient amount of aldrin has a noticeable effect in inhibiting subsequent locomotion by wireworms.

FIG. 2.—Count rate meter and Geiger-Mueller tube being used to determine the position of a tagged wireworm in an earthen crock.

When wireworms were released in crocks containing lindane-treated and untreated seeds, they usually were able to locate and feed on the untreated seeds. Also they seemed to avoid feeding on treated seeds or remaining in their vicinity for extended periods.

The behavior of larvae released in crocks containing dieldrin-, endrin-, and heptachlor-treated seeds, respectively, was too variable to make generalizations regarding relative lengths of visits to treated seeds.

Experiment II.—When the total minimum distances traveled by each larva during the last 3 days of the tests were averaged for larvae exposed to the same seed treatment, the average distances traveled per larva during this period were: lindane, 54; endrin, 39; control, 37; dieldrin, 32; heptachlor, 28; and aldrin, 15 inches. The ratio of the average distance moved during the first 3 days to the average distance moved during the last 3 days was 4.3 for larvae confined with aldrin-treated seeds. Comparable ratios for larvae exposed to seeds treated with dieldrin, lindane, heptachlor and endrin were 0.9, 1.0, 1.1 and 1.2, respectively. Larvae confined with the untreated control seeds (fungicide and sticker only) traveled on the average

Table 1.—Longest visits made by wireworms to the vicinity of each seed treatment. Experiment I, 1956.

Insecticide Seed Treatments	Larva No.	Largest Number of Consecutive Observations at Which Each Wireworm Was Observed at Each Treatment Position[a]			
		0	0.5	1	2
Aldrin	1A	—[b]	7	—	—
	2A	—	—	—	17
	3A	8	5	6	17
	4A	—	4	2	1
	5A	—	—	—	16
	Average	8.0	5.3	4.0	12.8
Dieldrin	1D	8[c]	2	3	2
	2D	29[c]	1	1	1
	3D	—	—	16	—
	4D	1	1	—	1
	5D	2	3	1	3
	Average	10.0	1.8	6.5	1.8
Endrin	1E	1	2	1	11
	2E	13[c]	2	2	2
	3E	5	—	5	8
	4E	8	2	—	9
	5E	—	—	—	—
	Average	6.8	2.0	2.7	7.5
Heptachlor	1H	1	—	—	7
	2H	1	10	7	2
	3H	46	—	1	—
	4H	—	7	2	3
	5H	5	1	—	—
	Average	13.0	6.0	3.3	4.0
Lindane	1L	15	1	—	5
	2L	—[c]	—	—	—
	3L	15[c]	6	5	3
	4L	1	—	4	9
	5L	15[c]	2	1	1
	Average	11.5	3.0	3.3	4.5

[a] Positions indicated by ounces of insecticide per bushel of seed.
[b] (—) indicates that the larva was never "observed" in the vicinity of seeds at this position, and this finding was not used in computing the averages.
[c] The larva fed on at least one seed at this position.

Table 2.—Longest visits made by wireworms to the vicinity of treated seeds. Experiment II, 1956–57.

Insecticide Treatment	Larva No.	Largest Number of Consecutive Observations at Which Wireworms Were Within Feeding Distance of the Seeds[a]
Aldrin	1A	2
	2A	3
	3A	2
	Average	2.3
Dieldrin	1D	1
	2D	3
	3D	9
	4D	13
	5D	6
	Average	6.4
Endrin	1E	6
	2E	18
	3E	1
	Average	8.3
Heptachlor	1H	24
	2H	7
	3H	4
	4H	4
	5H	7
	Average	9.2
Lindane	1L	1
	2L	4
	3L	2
	4L	5
	Average	3.0
Control (None)	1C	7[b]
	2C	16
	3C	23[b]
	4C	6
	Average	13.0

[a] Records are included only for the larvae which were observed within feeding distance of seeds at least once.
[b] This larva fed on at least one seed.

1.7 times as much during the first half as during the last half of these tests. However, three of four larvae exposed to untreated seeds remained within feeding distance of these seeds for considerable periods of time during the last 3 days of the tests, and two of these larvae fed on the seeds, probably during this time. It seems reasonable that a convenient source of favorable food reduced their urge to move about.

Table 2 shows the duration of the longest visit made by each wire-worm to the vicinity of seeds receiving various insecticide treatments. Larvae confined with insecticide-treated seeds usually did not remain near these seeds for extended periods of time, although random burrowing may have brought them within feeding distance frequently. This was particularly true of larvae confined with seeds treated with aldrin or lindane. Larvae confined with seeds receiving no insecticide treatment usually remained with these seeds for relatively long periods of time, and chance contact with untreated seeds was not often followed by speedy withdrawal from the seed area.

No wireworm feeding occurred in any of the crocks containing seeds treated with insecticide, while two out of four wireworms fed on control seed that had received no insecticide treatment.

Discussion.—Experiment I indicated that when wireworms were offered a choice among several seed treatments involving different insecticides and rates of application, including untreated controls, they often were able to successfully locate and feed upon untreated seeds. Also they seemed to simultaneously avoid feeding on or maintaining prolonged contact with seeds which had been treated with varying amounts of lindane. Their ability to detect and avoid aldrin-, dieldrin-, endrin- or heptachlor-treated seeds was not as apparent as was the case with the lindane treatments.

In spite of the fact that Experiment I indicated that

wireworms respond differently to lindane-treated seeds than they do to seeds treated with the other four insecticides tested, certain limitations were imposed on the interpretation of these results by the experimental design. The fact that each crock contained four seed treatments, three of which were potentially toxic to the insect, made an exact evaluation of the effects of any particular treatment on it impossible. Experiment II was designed with the hope of eliminating this limitation.

In Experiment II each insect had an opportunity to react to only one seed treatment. Some difficulty was encountered because of tags becoming detached during the course of some of the tests. This, together with the fact that the only records reported are for larvae which were observed within feeding distance of seeds at least once, accounts for the unequal numbers of larval records in table 2. In spite of these shortcomings Experiment II supplements and enlarges upon the information gained from Experiment I.

In both experiments the wireworms demonstrated their ability to avoid staying for prolonged periods in the vicinity of lindane-treated seeds. This ability was apparent in Experiment II with aldrin-treated seeds, although in Experiment I the larvae seemed unable to move away from seeds treated with 2 ounces of aldrin per bushel after contacting them. Both experiments indicate that aldrin had the most pronounced effect in inhibiting random movement of any of the insecticides studied.

The fact that the average distance traveled per larva during the last 3 days of the tests in Experiment II was much greater for larvae confined with lindane-treated seeds than for any other larvae may be indicative of an escape reaction on the part of these larvae.

No larvae in either experiment ever fed upon insecticide-treated seeds so far as could be determined. A total of eight larvae in the two experiments attacked untreated control seeds.

A comparison of the average lengths of life following these tests with larvae tagged with cobalt-60 but not exposed to treated seeds with that of larvae which had neither been tagged nor exposed to treated seeds indicated no harmful effects on the larvae due to the tagging. However while examining specimens from these tests after the tags had been removed, an unusual variety in shapes of the caudal segments was observed and several specimens were immediately sent away for positive identification. All were identified by Lane (1957) as *Melanotus communis* Gyll., but concerning the appearance of their caudal segments he wrote: "Evidently what you did to the wireworms with the isotope deformed or burned them sufficiently to alter the shape of the ninth segment decidedly." Radiation from the cobalt-60 tags or some other factor associated with the tagging process may have been responsible for this.

CONCLUSIONS.—(1) The repellency of certain insecticide seed treatments to wireworms apparently has two components: (a) inhibition of the feeding reaction, and (b) orientation of the insects away from treated seeds. For convenience these are referred to as type one and type two repellency, respectively. The phenomenon implied by type two repellency does not preclude contact with treated seeds, but infers that after a wireworm has reached the vicinity of treated seeds, it tends to remain near them for a length of time determined by the degree of type two repellency of the treatment.

(2) Aldrin, dieldrin, endrin, heptachlor and lindane seed treatments all appear to possess considerable type one repellency, i.e., they tend to inhibit the feeding reaction. Data to be presented in another paper indicate that the degree of this repellency varies with the insecticide used.

(3) Type two repellency was most pronounced with lindane-treated seed. It was also characteristic of aldrin-treated seed when only 0.5 or 1 ounce was applied per bushel. Two ounces of aldrin apparently gave an immediate toxic effect which inhibited the orientation reaction away from the treated seeds. Dieldrin, endrin and heptachlor seed treatments were characterized by a degree of type two repellency intermediate between lindane and aldrin on the one hand and untreated control seeds on the other.

(4) The technique of tagging wireworms with radioactive cobalt to follow their movements in the soil provides a useful method for making detailed studies of the movements of individual wireworms under more natural conditions than are otherwise possible.

(5) Certain practical implications of these studies will be discussed more fully in a future publication presenting additonal corroborative data. However the present report suggests that, comparatively speaking, aldrin deserves more consideration for use as a seed treatment than it has previously received.

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Susceptibility of Some Insect Pests to Infection by *Bacillus thuringiensis* Berliner in Laboratory Tests[1]

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ABSTRACT

The spore-forming bacterium *Bacillus thuringiensis* Berliner has demonstrated moderate-to-high pathogenicity for several leaf-chewing insect pests in southern California including larvae of the salt-marsh caterpillar, cotton leaf perforator, celery leaf tier, amorbia, bollworm, and larvae and adults of the Egyptian alfalfa weevil. Less susceptible were the cabbage looper, beet armyworm and omnivorous leaf roller. Only slightly susceptible were larvae of the alder flea beetle and larvae and adults of the elm leaf beetle. "Extra" mortality thought to be due to a toxic effect induced by the bacillus was noted in infectivity tests against some of the insect species.

Although economic entomologists have been displaying increasing interest in the use of microorganisms in the suppression of insect pests, and a number of reports of success in the microbial control of insects have appeared in recent years, widespread testing and use of these "living insecticides" have been retarded by the lack of these agents in commercial quantities. An upsurge of interest in the commercial production of the spore-forming bacterium, *Bacillus thuringiensis* Berliner, has followed a report by Steinhaus (1956) on the potentialities of microbial control of insects.

In anticipation of the possible availability of commercial supplies of *Bacillus thuringiensis*, a laboratory testing program was undertaken to determine the susceptibility of some of the important leaf-chewing insect pests in southern California to this organism. Only a limited number of species were tested, but the bacillus has demonstrated pathogenicity for several that are economically important. Some of these pests are becoming more difficult to control chemically because of developing resistance, or chemical control is undesirable because of residue problems. Laboratory successes often cannot be repeated in the field, however, where many uncontrolled factors influence the outcome of any biological action. The results presented here are therefore intended only to indicate the relative susceptibility of certain insect pests to *B. thuringiensis*. The results should serve as a guide toward subsequent field tests which may determine whether or not the bacillus can be economically used in the control of some of these insects.

MATERIALS AND METHODS.—The bacillus spores used in the infectivity tests were prepared in 1952 (from the Mattes strain of *B. thuringiensis*) by the method described by Steinhaus (1951). Without further refinement of the spore material the crystals were still present, and although this material was 5 years old, tests indicated that there had been no noticeable loss of viability of the spores. There were approximately 100 billion viable spores in each gram of dry powder.

In the laboratory experiments, the control and test insects were reared in replicate in petri-dish-covered, sterile half-pint cartons. The insects were fed suitable plant-leaf material held by cotton plugs in small vials of water. The test insects, which were either laboratory-reared or field-collected, were inoculated *per os* by feeding on leaves that had been thoroughly wetted in a suspension of bacillus spores. Where required, a wetting agent (Atlox 1045-A, Atlas Powder Company, Wilmington, Delaware; or Triton X-100, Rohm & Haas Company, Philadelphia, Pennsylvania) was used at the rate of 0.1 ml. per 50 ml. of a water suspension of infective material, except in one test in which a small amount of blood albumin was used. The food of the control insects was treated with a solution of the same wetting agent at the same concentration used for the test insects in each experiment.

A standard concentration of 1 gram of spore powder in 100 ml. of water was used in the initial infectivity tests. This concentration is roughly equivalent to $\frac{1}{2}$ pound of actual bacillus spore powder in 5 gallons of water. Higher concentrations of spore material were used in additional tests against insects that were found to be less susceptible to infection by the bacillus. In some instances lower spore concentrations were tested against insects that were highly susceptible to the organism at the standard dosage. Infection of the test insects by *B. thuringiensis* was determined by microscopic examination of all larvae that died during each 1-, 2-, or 3-day period. The presence of large numbers of readily discernible rods of *B. thuringiensis* in the cadavers of the test insects was used as the criterion for susceptibility. Results of the tests are presented in table 1.

RESULTS.—Larvae of the following insects were found to be at least moderately susceptible to *B. thuringiensis* in the laboratory tests: salt-marsh caterpillar, *Estigmene acrea* (Drury); cotton leaf perforator, *Bucculatrix thurberiella* Busck; celery leaf tier, *Udea rubigalis* (Guen.); tortricid leaf roller, *Amorbia essigana* Busck; bollworm, *Heliothis zea* (Boddie); and the Egyptian alfalfa weevil, *Hypera brunneipennis* (Boh.). The adults of the weevil also were found to be susceptible to the organism. Insects found to be less susceptible to infection by the bacillus at the standard dosage were the cabbage looper, *Trichoplusia ni* (Hbn.); the beet armyworm, *Laphygma exigua* (Hbn.); and the omnivorous leaf roller, *Platynota stultana* Wlshm. Larvae of the alder flea beetle, *Altica ambiens* (Lec.), and larvae and adults of the elm leaf beetle, *Galerucella xanthomelaena* (Schr.), were found to be only slightly susceptible.

A preliminary test of first-instar larvae of the omnivorous looper, *Sabulodes caberata* Guen., gave negative results when the test individuals refused to feed and starved to death. No signs of the bacillus were noted when the dead larvae were examined microscopically. The control larvae that were fed untreated plant material were

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Table 1.—Susceptibility of some leaf-chewing insect pests to infection by *Bacillus thuringiensis* Berliner in the laboratory.

Insect	Stage and Instar	No. of Insects Tested (Test/Control)	Dosage (Grams Spore Powder/100 Ml. Water)	Cumulative Per Cent Mortality (Days After Ingestion of Infective Material)[a]				
				2	3	4	6	16
Tortricid leaf roller, *Amorbia essigana* Busck	Larval (1)	36/16	1.00	100	—	—	—	—
	Larval (3)	15/5	1.00	100	—	—	—	—
Salt-marsh caterpillar, *Estigmene acrea* (Drury)	Larval (2)	25/25	0.25	76	84	—	—	—
	Larval (2)	30/30	1.00	77	93	—	—	—
Cotton leaf perforator, *Bucculatrix thurberiella* Busck	Larval (5)	25/25	0.25	36	56	56	64	—
	Larval (5)	50/50	1.00	94	—	100	—	—
Celery leaf tier, *Udea rubigalis* (Guen.)	Larval (1)	50/50	1.00	100	—	—	—	—
	Larval (5)	50/50	1.00	98	—	100	—	—
Cabbage looper, *Trichoplusia ni* (Hbn.)	Larval (2)	25/25	1.00	12	—	16	36	48
	Larval (3)	25/25	4.00	100	—	—	—	—
Egyptian alfalfa weevil, *Hypera brunneipennis* (Boh.)	Larval[b]	50/50	1.00	44	56	64	—	—
	Larval[b]	50/50	4.00	62	80	84	—	—
	Larval[c]	50/50	1.00	26	66	89	—	—
	Adult	50/50	1.00	—	10	—	40	—
	Adult	50/50	4.00	—	42	—	74	—
Beet armyworm, *Laphygma exigua* (Hbn.)	Larval (1)	25/25	0.25	32	36	44	—	—
	Larval (1)	25/25	2.00	28	36	36	—	—
	Larval (4)	25/25	0.25	0	12	12	—	—
	Larval (4)	25/25	2.00	20	48	52	—	—
Omnivorous leaf roller, *Platynota stultana* Wlshm.	Larval (4)	20/20	1.00	25	30	35	—	—
Bollworm, *Heliothis zea* (Boddie)	Larval (2)	25/25	1.00	52	—	—	—	—
	Larval (2)	25/25	4.00	56	—	—	—	—
Elm leaf beetle, *Galerucella xanthomelaena* (Schr.)	Larval (1)	25/25	0.25	16	—	20	—	—
	Larval (1)	25/25	2.00	16	—	20	—	—
	Larval (4)	10/10	1.00	0	—	0	—	—
	Adult	15/18	1.00	27	—	—	—	—
Alder flea beetle, *Altica ambiens* (Lec.)	Larval (4)	25/25	2.00	—	8	—	—	—

[a] Dash indicates that mortality was not determined or that culumative per cent mortality did not increase after last determination.
[b] Instar not known; larvae tested were half grown.
[c] Instar not known; larvae tested were mature.

normal in feeding and growth during the period of the test.

In infectivity tests against three of the insect species named, considerable mortality was noted that could not be attributed to the bacillus, since no signs of the organism were detectable. This unexplained mortality was striking because very little mortality was noted among the control insects. Microscopic examination revealed that the dead insects were filled with bacteria commonly associated with the gut of healthy insects rather than with *B. thuringiensis*. This "extra" mortality, which varied directly with the dosage of the test organism, was most noticeable in infectivity experiments against larvae of the beet armyworm and of the bollworm, and against adults of the Egyptian alfalfa weevil. Results of tests against these insects, including percentages of direct infection and of the "extra" mortality encountered, are presented in table 2.

It is evident from these data (table 2) that in some of the tests total mortality was much greater than that which could be traced directly to infection by *B. thuringiensis*. This "extra" mortality is thought to be due to a toxic effect induced by the bacillus. The percentages of mortality presented in table 1 are conservative in relation to those of the insects listed in table 2, and over-all effect of the bacillus is probably greater than shown.

DISCUSSION.—Because of the moderate-to-high mortality achieved in the laboratory tests, the possibility of microbial control of the salt-marsh caterpillar and of the surface-feeding stages of the cotton leaf perforator and the celery leaf tier in the field, utilizing commercially produced *B. thuringiensis*, is most certainly worth further investigation. Similar control of *Amorbia*, a major pest of avocado in California, is a possibility. Further laboratory testing is necessary, however, to ascertain whether less concentrated spore suspensions, more economical for

Table 2.—Results of infection of three species of susceptible insects by *Bacillus thuringiensis* Berliner, showing percentages of direct infection and of "extra" mortality attributed to toxic effect of the test organism.

Insect	Stage and Instar	No. of Insects Tested (Test/Control)	Dosage (Grams Spore Powder/100 Ml. Water)	Days After Ingestion of Infective Material	Cumulative Per Cent Mortality Following Ingestion of Infective Material					
					Control			Test		
					Direct Infection	Extra Mortality	Total	Direct Infection	Extra Mortality	Total
Laphygma exigua (Hbn.)	Larval (4)	25/25	0.25	4	0	0	0	12	20	32
	Larval (4)	25/25	2.00	4	0	0	0	52	44	96
Hypera brunneipennis (Boh.)	Adult	50/50	1.00	6	0	6	6	40	14	54
	Adult	50/50	4.00	6	0	6	6	74	20	94
Heliothis zea (Boddie)	Larval (2)	25/25	1.00	2	0	8	8	52	32	84
	Larval (2)	25/25	4.00	2	0	8	8	56	24	80

saturation treatment of avocados, will prove as effective as the suspension used in this determination.

The Egyptian alfalfa weevil is one of the two insect species in this group in which both the larvae and the adults are leaf feeders. It is interesting to note that the adults also are susceptible to the bacillus. The final larval mortality figures obtained are conservative, since the results were influenced by the presence of the parasite *Bathyplectes curculionis* (Thom.) in the field-collected weevil larvae used in the test. If the effects of the insect parasite had not entered the picture, the percentage of larval mortalities would have been more impressive. Since field results rarely approach those obtained in the laboratory, chances of successfully controlling economic infestations of the Egyptian weevil by the bacillus alone are slight. If the bacterium is capable of bringing about a marked reduction in larval and adult weevil population, since *B. curculionis* often parasitizes more than 30% of the weevil larvae, combination control by the two agents may be possible when the organism is used in areas where the parasite is giving partial control.

The results obtained in the cabbage looper infectivity test are similar to the findings reported by Tanada (1956). It is evident that the cabbage looper is only slightly susceptible to the bacillus at the standard laboratory dosage, but there is an indication that higher dosages may give satisfactory control. It is also possible that good kill of the cabbage looper on certain truck and field crops may be obtained by utilizing a mixture of *B. thuringiensis* and the polyhedrosis virus which has been reported by Hall (1957) to be effective against the looper on lettuce.

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Sour Cherries Infested with Butterfly Chrysalids[1]

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Reports of foreign objects attached to harvested sour cherrie were received during the 1957 canning season. The objects, thought to be of insect origin, were being frequently intercepted on the sorting belts during the canning operations at several large processing plants in Door County, Wisconsin.

Specimens received from the Fruit Growers Cooperative in Sturgeon Bay were identified as butterfly chrysalids. Several species were involved, one of which was the gold-flecked, jade green pupa of the monarch butterfly, *Danaus plexippus* (L.) (Fig. 1). The chrysalids were held for adult emergence. Several monarch butterflies later emerged, but, unfortunately, adults of the other species were not obtained.

The presence of the chrysalid infested cherries constituted a serious problem and necessitated additional vigilance to prevent their deposition in canned cherries. This problem was unique in the history of the sour cherry canning industry of Wisconsin.

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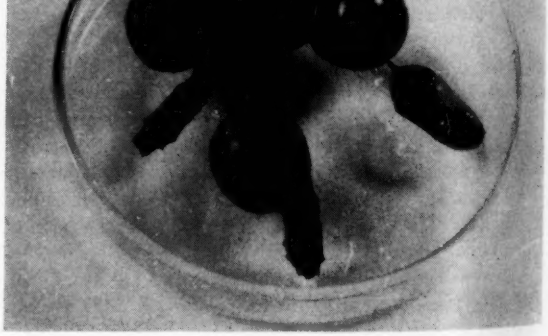

Fig. 1.—Butterfly chrysalids attached to Montmorency cherries.

Resistance of Some Rodenticidal Baits to Infestation by *Tribolium confusum* Duv.[1]

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Abstract

Thirteen materials, including warfarin and 11 indandiones were tested for ability to kill adults of the confused flour beetle (*Tribolium confusum* Duv.) and to prevent larval development in corn meal baits. DDT was used as a standard lethal material. All baits used were prepared by mixing the test material with white corn meal. The highest concentration of test material used in initial tests was 0.5%. Subsequent tests at lower concentrations were made using the compounds which had proven effective at the 0.5% level. Survivors were determined after beetles had been allowed to feed for 7 days in the bait mixture. Of the compounds tested, only Pival and DDT were consistently effective against adult beetles. No live beetles were recovered from any of the DDT bait mixtures. The Pival baits yielded 24% survivors at the 0.5% level and 84% survivors at the 0.05% level. None of the other compounds tested showed appreciable effect against adults. After the adults had been removed from each bait, the mixture was set aside for 7 days and then observed for larval development. No larvae could be found in any of the baits containing DDT, Pival, isovalerylindandione or acetylindandione at the 0.2% level. Propionylindandione and heptanoylindandione showed some inhibitory effect at the 0.5% level, but not sufficient to warrant testing at the lower level. All the other compounds tested (including warfarin) were ineffective in preventing development of larvae.

The widespread use of anticoagulant rodenticides has introduced a new problem into agricultural circles. Prior to the use of warfarin and Pival, rodenticides were acutely fatal and baits were not left around for long periods of time. However, one of the main advantages of the anticoagulant compounds lies in their low single dose toxicity compared with their relatively high multiple dose toxicity. This necessitates that the baits be left for feeding for several days. When the baits are kept adjacent to stored grain products, the possibility arises of introduction of grain pests such as the confused flour beetle (*Tribolium confusum* Duv.) which might be present in the rodenticidal baits.

The 2-acyl-1,3-indandiones such as Pival (2-pivalyl-1,3-indandione) and more recently Diphacinone (2-diphenylacetyl-1,3-indandione) (Saunders *et al.*) have been introduced as rodenticides following the report by Kabat *et al.* (1944) that some of the 2-acyl-1,3-indandiones discovered by Kilgore *et al.* (1942) were anticoagulant and toxic to rats as well as insecticidal. Of the various compounds studied by Kilgore and by Kabat, the most effective from both standpoints was 2-pivalyl-1,3-indandione. Field tests of this material by the Branch of Predator and Rodent Control, Fish and Wildlife Service, U. S. Department of Agriculture, disclosed that cereal baits prepared with Pival are less susceptible to insect contamination than baits containing warfarin. Lhoste & Leibovici (1954) have recently shown that baits containing 0.025% Pival are toxic to the adults of *Calandra granaria* and to the larvae of *Tribolium castaneum* (Hbst.) and *Ephestia kuehniella*, (Zell.) while Laudani *et al.* (1954) have reported that both Pival and Valone (2-isovaleryl-1,3-indandione) inhibit or prevent the development of *Tribolium* sp., *Lasioderma sericorne* (*F*)., *Oryzaephilus surinamensis* (*L.*), and *Ephestia* sp. in rearing tests using the rodenticides in concentrations of 0.025% in corn meal. In bait-station tests conducted in a room infested with 12 species of stored-product insects, these same authors state that while all baits tested had an equal attraction to the adult insects, the Pival and Valone baits contained "practically no larvae." In view of the potential value of insect-resistant baits, it was decided to test several rodenticidal baits for their resistance to infestation by *T. confusum*.

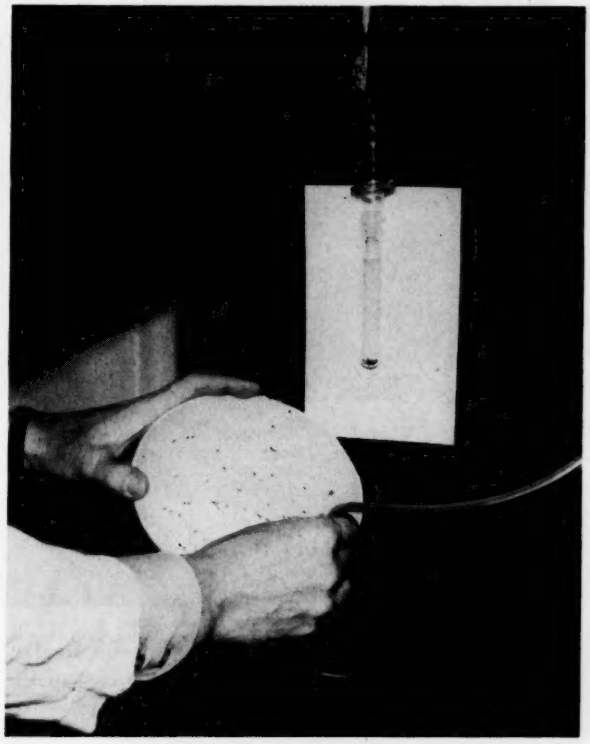

Fig. 1.—Adults of *Tribolium confusum* may be removed and counted with an aspirator, using controlled vacuum, as they ascend the interior of a cylindrical paper carton.

Procedure.—Warfarin, DDT and 12 indandiones[2] were tested for their ability to kill *T. confusum* adults, and to prevent larval development in corn meal baits. DDT was used in order to have some measure of the potential lethality of bait mixtures. All baits were

[1] Accepted for publication November 14, 1957.

[2] Eleven of the compounds used were 2-acyl-1,3-indandiones, with acyl groups as follows: pivalyl, piperidoyl, propionyl, isovaleryl, isobutyryl, heptanoyl, naphthoyl, acetyl, methyl benzyl, phenylacetyl, diphenylacetyl (diphacinone). The remaining compound was 2-phenyl-1,3-indandione.

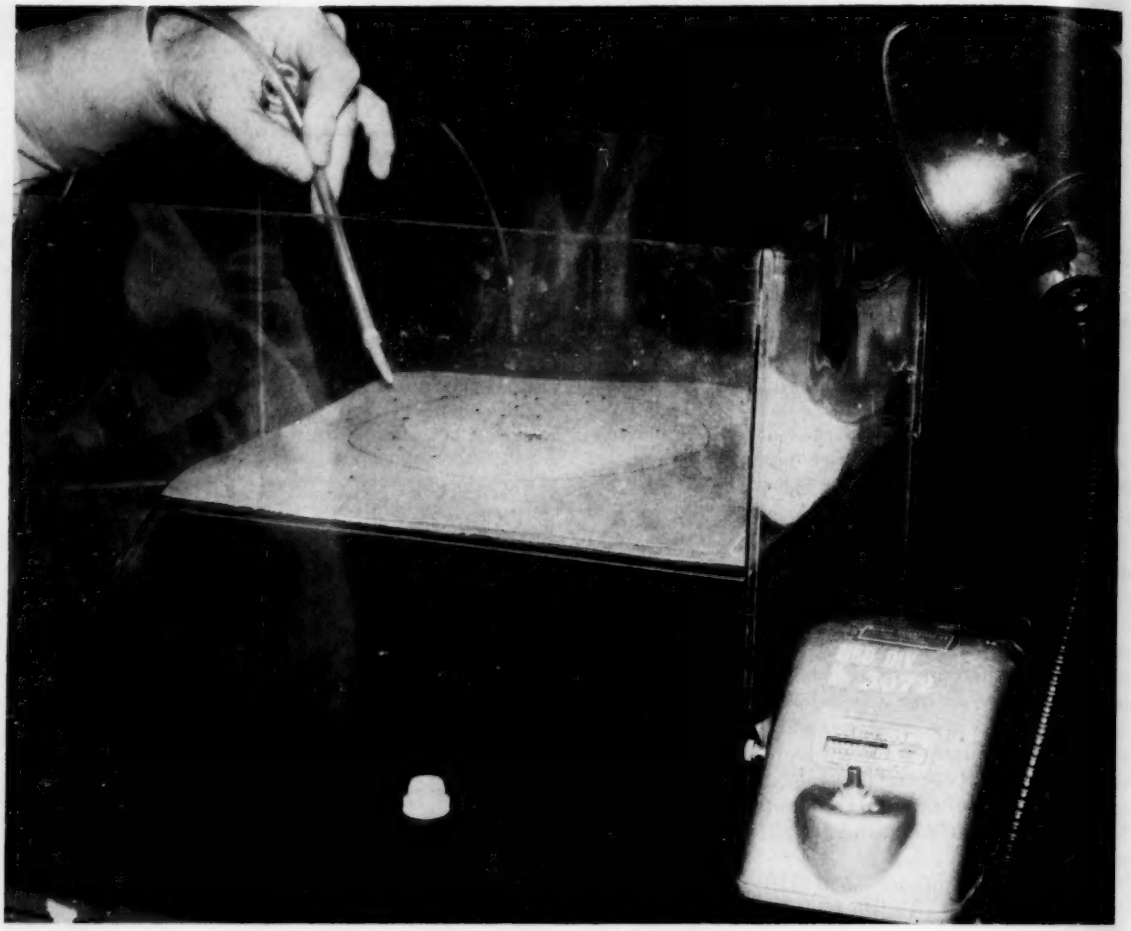

FIG. 2.—Method for determining survival of *Tribolium confusum*. Adult beetles are timed as they are placed in the center of the circle shown above. Those which have not left the circle within 100 seconds may be separated as dead or moribund.

prepared using white corn meal. The highest concentration of test material used in initial tests was 0.5%. Subsequent tests at lower concentrations were made using those compounds which had been found effective at the 0.5% level.

T. confusum were reared in a corn meal medium. Harvesting of adults was done by placing a portion of the rearing medium on the bottom of a 1-gallon cylindrical paper carton. The beetles immediately began to leave the medium and ascend the interior surface of the carton and were removed and counted by means of a one-eighth inch nozzle attacked to the collecting hose of a large mechanical aspirator. It was found that the velocity of air flow through the aspirator was important, it being necessary to adjust the vacuum so that the air flow was low enough to do no injury to the beetles. A removable collecting tube was fastened to the bottom of the aspirator. The technique is illustrated in figure 1.

Each bait was prepared by mixing the appropriate amount of test compound with 50 grams of white corn meal. Twenty five adult *T. confusum* were placed in 12 grams of each bait mixture. At the end of 7 days the bait was sifted through a No. 16 sieve and the adult beetles collected from the sieve by means of the aspirator. The beetles were then kept conveniently in 4-ml vials with perforated polyethylene stoppers. Counting of survivors was done by dumping the contents of a vial into the center of a 7-inch circle drawn upon a piece of brown wrapping paper which had been placed on the raised shelf of a transparent plastic bin beneath a bright light. A clock was started at the moment the beetles were introduced into the circle. Control studies on normal beetles under these conditions had shown previously that all beetles leave the circle in less than 50 seconds. An arbitrary time limit of 100 seconds was then designated for survival determinations. All beetles remaining within the circle at the end of the time limit were counted as dead or moribund. This technique is illustrated in figure 2.

Two weeks after the start of each experiment the effect of each compound on larval development was studied. This was done by evenly distributing the bait mixture over the bottom of a 6-inch petri dish and examining it beneath a dissecting microscope. Ten 15× fields were carfully probed with a beveled applicator stick, and the larvae found therein counted. Larval development was recorded as none, rare, few, moderate, numerous, or very numerous depending on the number of larvae found in ten 15× fields.

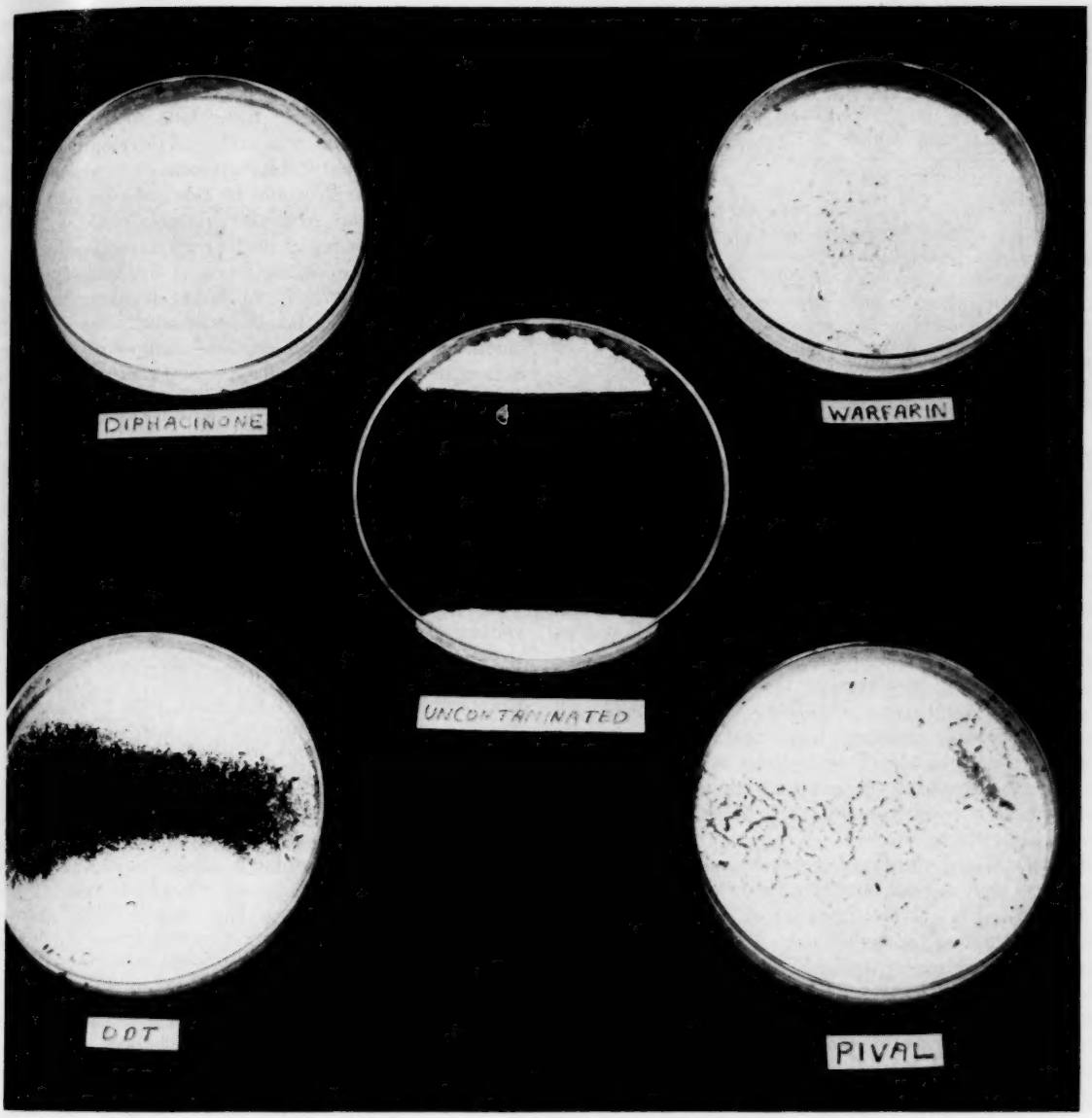

FIG. 3.—Feeding preference shown by *Tribolium confusum*. At the start of the experiment each petri dish appeared as the one in the center, marked control. The piles of corn meal shown at the bottom of each dish were untreated and those at the top were treated with the compounds labeled beneath. Fifty beetles were placed in the center of each dish. Tests are shown above as they appeared after 5 weeks.

An experiment to determine the repellency to *T. confusum* of 0.5% baits prepared from corn meal and DDT, warfarin, pival or diphacinone was carried out as follows. Two 5-gram piles of corn meal, one treated and the other a control, were placed at opposite ends of a 6-inch petri dish. Fifty beetles were then placed between the piles of corn meal which were approximately 4-inches apart. The dishes were covered and set aside for several weeks. At the end of 5 weeks the piles were examined for intermingling. This technique is illustrated in figure 3.

RESULTS.—Of the compounds tested for ability to kill *T. confusum* adults, only DDT and Pival were consistently effective. DDT proved to be 100% effective against adults at concentrations as low as 0.2% but began to lose some of its effectiveness below this concentration. Pival, on the other hand, gave variable results. At 0.5% bait concentration only 24% live adults were recovered, whereas at the 0.1 and 0.05% levels approximately 51 to 84% live adults were recovered. None of the other compounds tested showed any appreciable effect against adult beetles. A summary of the results obtained with the active compounds is given in table 1.

Several of the compounds ineffective against live beetles were quite effective against larvae. No larvae could be found in any of the bait containing DDT, Pival, isovalerylindandione or acetylindandione even in baits containing only 0.2% of the compound. Propionyl-and heptanoyl-indandione showed some inhibitory effect at

Table 1.—Effect of bait mixture on adult *Tribolium confusum* after 7 days feeding.

Bait Mixture of Corn Meal	Number of Adults		Per Cent Recovery
	Added	Recovered Alive	
Pure corn meal	225	222	99
0.5% DDT	125	0	0
0.3% DDT	50	0	0
0.2% DDT	40	0	0
0.5% Pival	174	42	24
0.3% Pival	48	41	85
0.2% Pival	50	41	82
0.1% Pival	51	26	51
0.05% Pival	49	41	84
0.1 DDT	49	6	13
0.05 DDT	50	8	16

the 0.5% level, but not sufficient to warrant testing at lower levels. All other compounds tested were ineffective in preventing development of larvae.

Results of the repellency tests are indicated in figure 3. Within 8 days of the start of the tests, a distinction between the amount of working in treated and that in untreated corn meal could be seen in the DDT and Pival tests. The distinction was greatest in the Pival test in which at that time no dead or moribund beetles could be observed. Several beetles near the DDT-treated baits, on the other hand, were seen to be dead or moribund. This fact probably explains why there was less working in the controls for the DDT tests than in those for Pival tests. Baits containing warfarin and Diphacinone showed equal working in control and treated corn meal. After 5 weeks, little change had occurred in the test dishes containing DDT. In the Pival tests, the untreated piles of corn meal were spread out to coincide with the margins of the treated piles which themselves showed little more spreading than on the eighth day. A few dead and moribund beetles were seen in the Pival tests at the end of 5 weeks. In the Diphacinone and warfarin tests, the treated and the untreated piles could not be distinguished after 5 weeks, the beetles having worked both piles into an even dispersion over the bottom of each dish.

DISCUSSION.—The only anticoagulant compound that showed appreciable activity in killing adult *T. confusum* was Pival. This effect was seen in baits containing from 0.05 to 0.5% of the rodenticide, although in the lower concentrations, the effect was not very great when compared with the mortality produced by similar concentrations of DDT. Further, at the 0.05 to 0.5% levels of Pival-corn meal, there was no larval development. These findings indicate that stock corn meal bait, which usually contains 0.5% Pival, would be relatively immune to infestation by *T. confusum*, and probably could not, therefore, serve as a source of beetle contamination for stored grain and grain products. Even in final-use type baits containing only 0.05% Pival, larval development can no longer proceed, even though some adults survive in this medium. Another anticoagulant compound, isovalerylindandione, showed no effect against adult beetles, but did retard larval development. The probability is that baits prepared with this material would not serve as a source of contamination.

Two other anticoagulant rodenticides, warfarin and Diphacinone, neither repelled or killed adults, nor did they prevent growth of larvae. Thus baits prepared from either of these two materials could become infested with *T. confusum* and serve as potential sources of contamination for stored grain where such baits are used for the control of rodents.

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NOTICE TO AUTHORS

Each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY must be accompanied by an abstract suitable for publication, and prepared in accordance with instructions for the preparation of abstracts as required by *Biological Abstracts*. This abstract will replace the usual author's summary. See "Important Notice to Authors" which appeared in the *Bulletin of the Entomological Society of America* 3(2): 1. June, 1957.

A Method of Rearing Large Colonies of an Eriophyid Mite, *Aceria tulipae* (Keifer), in Pure Culture from Single Eggs or Adults[1]

MARIA SALOME DEL ROSARIO AND WEBSTER H. SILL, JR.[2]

ABSTRACT

A successful method of rearing large colonies of an eriophyid mite, *Aceria tulipae* (Keifer), vector of wheat streak mosaic virus, from a single mite or egg under greenhouse conditions was devised by using a series of different cages as early colonizing chambers. The cages in the order of their use were (1) petri dish, (2) large test tube plugged with rubber sponge and inverted in a complete nutrient solution, (3) lamp chimney set in 4-inch flower pot, (4) several types of larger cages of more standard design. Each cage was designed so that eriophyid mites could not gain voluntary or chance entrance or exit. Whole plants bearing mites were transferred from one cage to the next larger one when growth conditions for plants or mites became unfavorable. A high humidity was necessary for rapid increase in mite populations, particularly when colonies were being established. The mites were transferred successfully in three ways. Since they proved to be negatively phototropic, it was possible to herd them by light, either incandescent or flashlight. Mites transferred in this way moved voluntarily and were never injured although it was difficult to control the number moving. A medium-coarse human hair was the most useful tool for transferring single mites and eggs. The hollow end was used for picking up eggs and the pointed tip for moving nymphs and adults. As the third method portions of mite-infested leaves were inserted into the youngest leaf axils of healthy plants and kept in place with a woman's-hair clip. This method was not very practical as mites often did not migrate and died on the excised leaves. Also, it was very difficult to determine the time of migration and the number of mites migrating to the new host. High humidity in the micro-environment may be an important factor in the establishment of large mite populations in the field. Although mites thrived best in the greenhouse under high humidity conditions, evidence indicated that prolonged flooding (24 hours or more) was detrimental and eventually lethal.

Slykhuis (1953) first reported the occurrence of *Aceria tulipae* (Keifer) on wheat plants. He also demonstrated the ability of this mite to transmit wheat streak mosaic virus. In transmission studies of strains of wheat streak mosaic virus by *A. tulipae* (Lal *et al.* 1957), attempts to rear them individually under controlled conditions using techniques reported by Slykhuis (1955) and Staples & Allington (1956) were often unsuccessful. *A. tulipae* is a very small mite, about 270μ in length. It is not visible to the naked eye when occurring singly, and must be magnified at least 20 to 50 times to be studied, and is too small to rear in pure culture using standard insect cage techniques. Hence, it was necessary to devise a method of rearing and handling individual mites and their progeny under controlled conditions. This paper reports a technique which has been very successful and includes a discussion of certain mite habits that have been observed during the course of the study.

MATERIALS AND METHODS.—Mites were obtained from naturally infested wheat plants in the field. It was necessary to obtain nonviruliferous mites at the start, and since eggs do not transmit the virus (Slykhuis 1955), only newly laid eggs were used. These were moved singly with medium-coarse human hair to healthy wheat plants. The newly hatched nymphs were then transferred to other healthy and diseased wheat plants as needed. When necessary, mites were sent to H. H.Keifer[3] for specific identification.

Greenhouse Procedures.—All wheat plants were grown from seed in steam sterilized soil and in cages described later in this paper. Another section of the greenhouse where no mite experiments were in progress was used as a starting room for other uncaged wheat plants used occasionally. This starting room was given a preventive fumigation with Plantfume 103 (a smoke generator, active ingredient tetraethyl dithiopyrophosphate, 15%) each week and was kept entirely closed by an automatic ventilator and heating system during the winter. The daily temperature of the whole greenhouse was near 70° F. during the winter, but in the summer months ventilators had to be kept open and the temperatures were often 100° F. or more. The cement floor and wooden benches were constantly moistened during hot periods to maintain high humidity and to cool the rooms. The greenhouse roof was covered with a shading compound. The side ventilators were screened with 36-mesh lumite screen which excluded larger insects, such as aphids, but not mites.

Rearing Cages.—Five different types of cages were used successfully. The first was a large test tube, 2 cm. in diameter and 8 in. long, plugged with a synthetic rubber sponge stopper (fig. 1, B) and inverted in Hyponex plant nutrient solution (Hydroponic Chemical Inc.) 75 gm. to a gallon of water. These were used as starting cages.

The second cage consisted of an ordinary lantern globe or lamp chimmey covered on top with nylon taffeta which was pasted on the top opening with Armstrong caulking compound and held in place by two rubber bands (fig. 1, C). The bottom of the globe was then pushed into the soil around the growing plants.

The third cage was developed and illustrated by Fellows & Connin (1952) and consisted of ground-edged pyrex cylinders, 8 in. in diameter and 18 in. high with plastic tops set in Armstrong caulking compound. These cages were arranged eight in a row and cemented to a wooden bench. Underneath the bench a series of rubber manifolds, 1½ in. in diameter, was provided, one for each cylinder, and attached to a master manifold of galvanized iron which was connected to a Dayton fan blower with a capacity of 150 c.f.m. Each cage had an individual air in-

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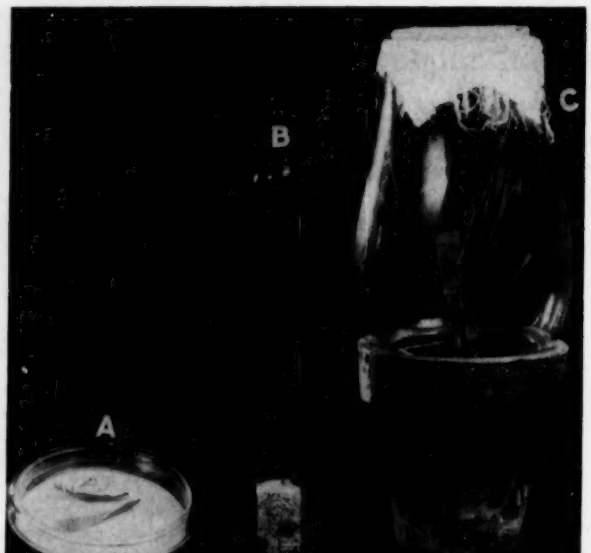

FIG. 1.—Early colonizing cages for rearing eriophyid mites, *Aceria tulipae* (Keifer). (A) Sterile petri dish lined with moistened filter paper used as a temporary cage. (B) Large test tube plugged with synthetic rubber sponge stopper and inverted in a complete nutrient solution. (C) Lamp globe cage pushed into the soil and covered with nylon taffeta, held in place with two rubber bands.

let and outlet, each covered with 36-mesh lumite screen. The air inlet attached to the rubber manifold had a hand-operated valve to control the flow of air. The pots in the cages were watered from below by means of brass and rubber tubings (opened and closed by pinchcocks) which were connected to a reservoir of water set high enough so that a gravity flow system to each pot operated when the pinchcocks were opened.

A wooden frame cage with dimensions of 18×24 in. and covered on three sides with nylon taffeta was used as the fourth type (fig. 2). A plastic sheet having three orifices closed by rubber stoppers served as a front. These cages were fitted tightly to 6-inch clay flower pots by means of a 6-inch diameter groove which was filled with Armstrong caulking compound. This cage is similar to those used in the Department of Plant Pathology, University of Nebraska.

The fifth cage was larger, made of glass with a wooden frame measuring 33×31×23 in. It also was reported and illustrated by Fellows & Connin (1952). It had a wood bottom and one wood end. Two orifices, 2 in., in diameter, were located in the wood end near the top; one, the air inlet, the other, for watering plants. The air inlet was covered with a 36-mesh lumite screen and the water opening was plugged with a 2-in. rubber stopper. Air was supplied by blower, described previously, through a tube of galvanized iron, 27 in. long, with a brass casting. The end of this galvanized iron tube, with 12 air inlet apertures, was inside the cage. The cage opened at the top by means of an 11×31 in. wooden door hinged to the frame.

Mite Handling Techniques.—Initially, eggs from field infested plants were placed on healthy excised wheat leaves in sterilized petri dishes lined with moistened filter paper (fig. 1, A). Leaves were examined twice daily for hatched eggs. The newly hatched nymphs were then transferred to 2-week-old wheat plants growing in the inverted test tube cage (fig. 1, B). On each leaf the location of each nymph was marked with India ink to facilitate future examinations. After 48 hours in the inverted test tube cages, the plants upon which mites were thriving were transplanted to the lamp globe cages (fig. 1, C). Plants were then examined under a binocular microscope twice a week for nymphs, adults, and eggs and were grown in these cages until good colonies were established. Then the plants carrying the mite colonies were transplanted to and maintained in the nylon taffeta or cylindrical glass cages already described.

RESULTS.—*Rearing Mites.*—Various techniques for rearing individual mites described by others (Slykhuis 1955, Staples & Allington 1956) were attempted with

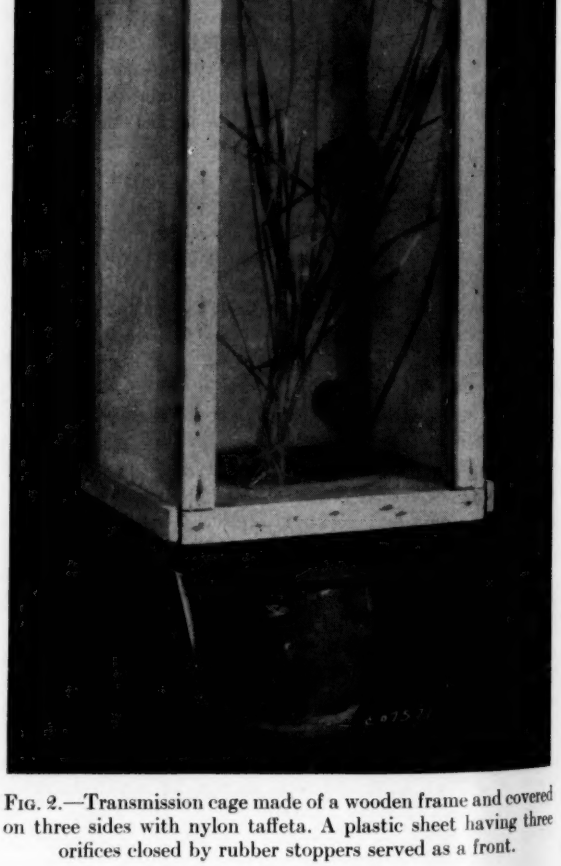

FIG. 2.—Transmission cage made of a wooden frame and covered on three sides with nylon taffeta. A plastic sheet having three orifices closed by rubber stoppers served as a front.

little success. Eggs hatched well but the majority of the nymphs when transferred to plants in the large nylon taffeta (fig. 2) or cylindrical glass cages did not survive. In an attempt to find a more favorable environment for the newly hatched nymphs, wheat plants were grown for 1 week in the inverted test tubes (fig. 1, B) and then were infested with the newly hatched nymphs. Nymphs were able to live in these tube cages more than 2 days, but because of the condensation of much moisture inside the tubes and the consequent soaking of the leaves, the mites often appeared to have drowned. Gibson (1957) reported that dislodged mites could survive at least 30 min. in water. This would suggest that the mites may have been drowned only after many hours of submersion on the condensed water. The cotton plugs used at first were eliminated and synthetic rubber sponge plugs were substituted to reduce condensation. A comparison of the two (table 1) showed that survival in the latter was much better. The cotton plugs permitted excessive flow of water into the test tubes, thus the base of the youngest leaf axil, where the mites usually were placed, was often filled with water. Even when rubber sponge stoppers were used, the tubes were changed twice a day or as needed to control the condensation. This method provided a fairly constant high humidity with a minimum of flooding. After a few days the plants in the test tube cages were transplanted to lamp globe cages (fig. 1, C) and maintained at a high humidity comparable to that in the test tube cages. When these plants developed trapped leaves and good colonies of mites and became too big for normal development in the lamp globe cages, they were transplanted to the larger nylon taffeta or glass cylinder cages and again maintained at high humidity. This method of rearing the mites was consistently successful.

Transferring Mites.—Various methods of transferring mites were tested. The three listed below were most valuable.

1. Transfer by light. By chance a negative phototropic mite response was noticed. When a 50-watt incandescent light was directed at them from about 5 inches, the mites consistently moved away from the light. This was also true with cold light furnished by a powerful flashlight. This made it possible to move or herd small groups of mites. Leaves covered with mites were placed upon leaves of uninfested plants. While constantly observing the mites under a binocular microscope magnifying 40×, light was directed at them. A definite number could be forced to migrate onto the uninfested leaves in this way without danger of injuring the mites as the movement was voluntary. This method was valuable for moving small groups of mites but was of no value for transferring a single mite.

2. Transfer by the use of human hair. Eggs were transferred easily under the microscope by using medium-coarse human hair with a hollow tip, while the adults and nymphs were moved with hair having pointed ends. To prevent injury of the mouth parts only mites that were moving were picked up. Medium-coarse human hair was consistently the best of the various bristles and hair tried and considerably better than the squirrel hair used by Slykhuis (1955).

3. Transfer by movement of infested leaf portions. Wheat leaves were examined for mite colonies. When a sufficient number of mites was located, the leaf was cut with a pair of scissors and the cut portion inserted with a pair of forceps into the youngest leaf axil of an uninfested wheat plant and clamped on with a woman's hair clip. This method was not very useful as mites often would not move to the new plant but remained and died on the excised leaves. Staples & Allington (1956) used a method very similar to this.

Table 1.—Comparison of cotton and synthetic rubber sponges as plugs in the inverted test tube cages.

Trial No.	No. of Mites Placed[a]	Number of Mites Surviving After 3 Days	
		Cotton	Rubber Sponge
1	5	0	5
2	10	0	9
3	2	0	2
4	2	0	2
5	7	0	6
6	6	0	6
7	3	0	2
8	1	1	1
9	1	0	0

[a] Individual mites transferred by human hair.

Discussion.—Successful rearing of large colonies of *Aceria tulipae* in pure culture from a single egg or mite was accomplished consistently by keeping mites in a humid environment, particularly while colonies were being established, and by transplanting whole infested plants where possible rather than moving individual mites. High humidity was maintained by using petri dish, test tube, and lamp globe cages, as early hatching and colonizing chambers. After colonies were established and mites were abundant, high humidity, although desirable, did not appear to be critical. High humidity in the microenvironment may be a factor in the establishment of large mite populations in the field, but information concerning this is still sparse. Although mites thrived best in the greenhouse under high humidity conditions, evidence indicated that prolonged flooding of 24 hours or more was detrimental and eventually lethal.

Various methods of transferring individual mites suggested by others either failed or were only partially satisfactory. Various types of hair were tested and medium-coarse human hair was found best in handling both individual eggs and mites.

The mites were negatively phototropic and moved away when either a bright incandescent light or light from a flashlight was directed at them at a range of 5 inches or less. Groups of mites could be moved readily by this method and were not injured during the transfer as they moved voluntarily. This method was relatively slow, however, and it was difficult to count the number of mites transferred.

Transfer by inserting a portion of a mite infested leaf into the leaf axil of an uninfested wheat plant was not very effective. The mites for unknown reasons would not always move actively from the excised host tissue to the new wheat plant; consequently, the mites often died in place when the transferred leaf portion of the host withered. Here again, it was difficult to determine the exact number of mites that migrated to the new host.

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Residue Studies of DDT and Malathion on Turnip Tops, Collards, Snap Beans and Lettuce[1]

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ABSTRACT

Analyses of turnip tops, sprayed three times with malathion at the rates of 10.1, 20.2 and 30.3 ounces active ingredient per acre per application, showed initial deposits of 1.3, 2.1 and 4.5 p.p.m., respectively. Collards sprayed twice with malathion at 16 ounces active ingredient per acre, showed residues no greater than 3.9 p.p.m. 3 days after the last application. Two applications of DDT at 16 ounces active ingredient per acre, left residues of 31.6 and 18.4 p.p.m. on collards and lettuce, respectively. The DDT residue on collards dissipated rapidly, and, by the 14th day, was below the federal tolerance of 7.0 p.p.m. The DDT residue on lettuce declined at the rate of approximately 1.0 p.p.m. per day and was also below tolerance at the end of 14 days. Snap beans sprayed twice with DDT at 12 ounces active ingredient per acre, showed an average residue of 0.8 p.p.m. 1 day after the last application.

It is a known fact that spray residues on plants tend to decrease with the passage of time and experience has shown that some have a tendency to disappear more rapidly than others. Among the factors involved is the ratio of surface area to mass. Ashdown & Watkins (1948) applied dusts in various concentrations and found less than 1 p.p.m. of DDT residue on the lettuce heads when the applications were stopped 2 to 3 weeks prior to harvest. They also found, when following this type of schedule, that most of the harvest residue was on the trimmings and only nominal amounts on the marketable heads.

Several workers have reported the results of residue analyses at harvest time following DDT application to cabbage. Carter (1948) found that the four wrapper leaves contained 6 p.p.m. DDT while the stripped heads had less than 0.5 p.p.m. Data reported by Manalo *et al.* (1946) revealed that 21 days after an application of 5% DDT dust the residue on the outermost mature leaves of cabbage was nearly four times as great as the residue on the heads. Reiber & Stafford (1946) took cabbage samples for analyses 36 days after two applications of 3% DDT dust, and reported residues of 4.2 p.p.m. DDT on the outer leaves and 1.1 p.p.m. on the inner leaves.

The decrease in arsenical residue was shown by Hamilton (1929) to be proportional to the rate of increase of the leaf area. It was shown from data on the increase in surface area and volume of apples that growth was more important in the reduction of residue when it increased rapidly, but when growth was slow, weathering was more important.

Residues of malathion resulting from applications on vegetable, berry and tobacco crops were reported by Wallis *et al.* (1957). It was shown that malathion residues were higher and remained longer on leafy vegetables and tobacco than on berries and fleshy vegetables. In comparing residues from different formulations they found emulsions were reduced the fastest.

Information on some of the more common leafy vegetables grown in Florida seemed to be scarce. This study was undertaken because considerable concern was expressed over the amounts of residue which might be present on these leafy crops at harvest time.

METHODS AND MATERIALS.—All crops were planted in 4-row plots with a Planet Jr. No. 300 hill and drill seeder. The rows were spaced 38 inches apart and the crops were grown under standard cultural methods as practiced in Florida. The sprays were applied with a John Bean Hi-Lo sprayer, which had been modified by having the standard 100-gallon spray tank removed and a steel rack installed upon which six 10-gallon milk cans were carried. The sprays were mixed in and pumped directly from the milk cans. One can filled with water was used to flush the line when changing from one spray to another. The sprayer was operated at a pump pressure of 200 pounds per square inch when used on snap beans, and at 100 to 130 pounds on turnips, collards and lettuce. The spray boom had three *Teejet* disc-type nozzles per row arranged as follows: one nozzle directly over the row and one at each side of the row directed downward at an angle of 45 degrees from the horizontal. The spray was applied at the approximate rate of 100 gallons per acre.

Three applications of 50% malathion emulsifiable concentrate were made to Shogoin turnips in 1953 at rates of 1, 2 and 3 pints per 100 gallons per acre per application. Two applications of 25% DDT emulsifiable concentrate were applied in 1955 to Contender snap beans at the

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Table 1.—Residues of DDT and malathion (p.p.m.)[a] found on turnip tops, snap beans, collards and lettuce in Florida, 1953–56.

Crop	Ounces of Active Ingredient per Acre per Application[b]	Time Elapsed After Last Application[c]					
		4 Hrs.	24 Hrs.	2 Days	3 Days	7 Days	14 Days
Turnip tops	*Malathion*						
	10.1	1.3–1.5	1.0–1.2	0.8–1.1		0.6– 0.9	
	20.2	1.8–2.3	1.7–2.2	1.0–1.7		0.9– 1.1	
	30.3	4.1–5.0	3.2–3.8	2.5–3.1		0.9– 1.3	
Snap beans	*DDT*						
	6.0		0.8–1.2		0.3– 0.4	0.1– 0.3	
	9.0		0.6–1.0		0.3– 0.5	0.2– 0.3	
	12.0		0.8–0.8		0.7– 0.8	0.4– 0.5	
Collards	*DDT*						
	8.0				14.8–20.1	7.1–10.0	1.3–1.9
	16.0				28.8–34.5	11.9–18.6	4.6–6.7
	Malathion						
	8.0				0.7– 1.7		
	16.0				2.6– 3.9	1.1– 1.9	
Leaf lettuce	*DDT*						
	16.0				17.2–19.6	13.2–15.7	5.9–8.5

[a] Expressed as the range of three replications.
[b] Dates of application: turnips—Nov. 28, Dec. 5 and 10, 1953; snap beans—May 20 and 30, 1955; collards and lettuce—Feb. 2 and 10, 1956.
[c] Dates of chemical analyses of sample extracts: turnips for malathion—Dec. 19, 1953–Jan. 11, 1954; snap beans for DDT—June 6–8, 1955; collards for DDT—March 8–14, 1956; lettuce for DDT—March 13–14, 1956; collards for Malathion—March 3–4, 1956.

rates of 1½, 2 and 3 pints per 100 gallons per acre per application. In 1956, two applications of 25% DDT emulsifiable concentrate were applied to Georgia collards at the rates of 1 and 2 quarts per 100 gallons per acre per application and 50% malathion emulsifiable concentrate was applied at 1 and 2 pints per 100 gallons. Also in 1956, 25% DDT emulsifiable concentrate was applied to Simpson black-seeded leaf lettuce at the rate of 2 quarts per 100 gallons per acre. Each of the above treatments was replicated three times and each block contained a check plot.

Sampling and Analysis.—Turnip plants which had been sprayed with malathion were cut at random from each plot. They were mixed thoroughly and a 600-gram (fresh weight) sample was extracted with 750 ml. of carbon tetrachloride. Two 250-ml. aliquots were placed in sealed Mason jars and stored at 40° F. until analyzed. DDT-treated snap beans were sampled by picking 10 to 15 pounds of beans from each plot. Beans were picked from as many plants as possible in the plot. An aliquot of 1000 grams was taken from each plot sample and extracted with 1000 ml. of hexane. Collard plants were cut at random from 4-row plots. The leaves were chopped into small pieces and mixed thoroughly. An aliquot of 1000 grams was taken from each plot sample. Collards which had been sprayed with DDT were stripped with 1000 ml. of hexane and those sprayed with malathion were stripped with 1000 ml. of methyl chloroform. Head lettuce which had been sprayed with DDT was cut at random from 4-row plots. The heads were then chopped into small pieces, mixed thoroughly, and a 1000-gram sample extracted with 1000 ml. of hexane.

Residue extractions were started about 1 hour after harvesting samples by tumbling with a solvent for 1 hour on horizontal rollers rotating at approximately 32 r.p.m. After extraction 300-ml. aliquots were saved from collards, snap beans, and lettuce extracts and stored at 40° F. until analyzed. Duplicate aliquots were saved in all experiments. The DDT-treated samples were analyzed using the modification by Downing & Norton (1951) of the basic Schechter-Haller colorimetric procedure. Malathion residues were determined by a colorimetric technique published by Norris *et al.* (1954).

RESULTS.—The interval between stripping and analysis and the results of the analyses are shown in table 1. Malathion residues on turnip tops and collards were very low. Figure 1 shows that the residues did not closely approach the established federal tolerance of 8.0 p.p.m. Turnip top samples treated with 30.3 ounces active ingredient per acre per application and taken 4 hours after the last spray application contained residues no higher than 5 p.p.m. While malathion has not yet been registered for use on collards, it is assumed that when and if it is, the tolerance will at least equal the 8.0 p.p.m. established for other crops. It would seem that under normal practice, excessive malathion residues would not be encountered on turnip tops and collards, or on other related leafy vegetables.

DDT residues on collards, and lettuce, sampled 3 days after the last spray application, were well above the federal tolerance of 7 p.p.m. DDT residues on snap beans sampled 1 day after the last application, were well below the tolerance. While DDT residues on collards were high, the reduction between the third and seventh day was quite rapid. The rate of reduction between 7 and 14 days was more gradual, but on the 14th day residues were below the tolerance level. DDT residues on lettuce declined at a somwhat more gradual rate, approximately 1 p.p.m. per day, from the third to the 14th day, but were also below the tolerance at the end of 14 days, figure 2. The low residues on snap beans may be explained, in part, by the ratio of surface area to mass. A bean pod has a small surface area in comparison to its total weight and the calculated residue would undoubtedly be lower than if

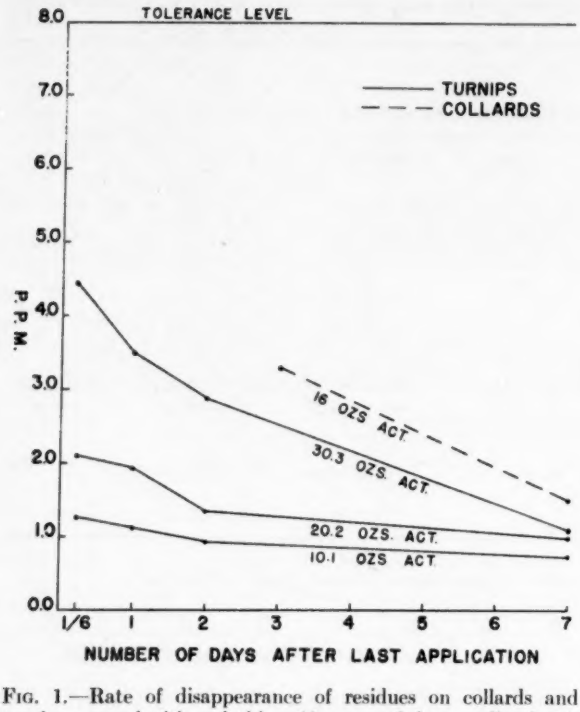

FIG. 1.—Rate of disappearance of residues on collards and turnips sprayed with malathion. (Average of three replications.)

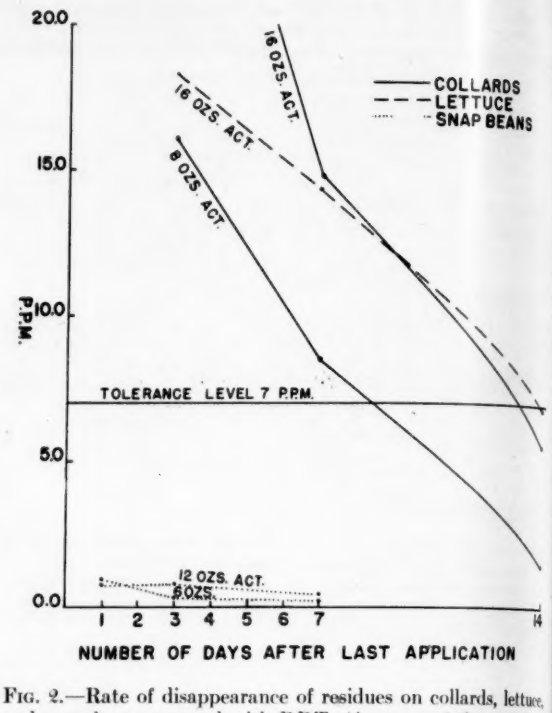

FIG. 2.—Rate of disappearance of residues on collards, lettuce, and snap beans sprayed with DDT. (Average of three replications.)

a leafy vegetable of the same green weight had been involved. It is quite important that sufficient time elapse between the last spray application and harvest on the leafy vegetables, which have a large surface area as compared to the mass, in order to allow for the dissipation of DDT residues. Snap beans were included in this study so that a non-leafy vegetable could be compared with a leafy one.

As these residue studies were conducted during different years on different crops it is difficult to correlate the effect of weather factors with the amounts of residue remaining on the crops. The average mean temperature was 78.6° F. in 1955 during the period the beans were under study but 15 degrees lower in 1953 when turnips were used and in 1956 when collards and lettuce were tested. Rainfall recorded from the last spray application to the last harvest was 2.39 inches on turnips in 1953 and 0.79 inches on collards and lettuce in 1956. There was no rain on the beans in 1953. The major factor in determining the amount of initial residue deposit is the rate of insecticide application. As a rule, the residues immediately after spraying and, generally, at any given interval after spraying, are closely related to the rate of application. This can be noted along with the comparative rate of reduction in malathion on turnip tops (fig. 1) and DDT on collards (fig. 2). Collards sprayed with DDT at the rate of 16.0 ounces active ingredient per acre and sampled 3 days after application contained an average residue of 31.5 p.p.m., while DDT applied at the rate of 8.0 ounces of active ingredient per acre showed an average residue of 16.6 p.p.m. A distinct disappearance slope was obtained for each rate of application. When malathion was applied to turnips at the rates of 10.1, 20.2 and 30.3 ounces active ingredient per acre, analyses showed initial deposits of 1.29, 2.10 and 4.48 p.p.m., respectively. As subsequent residues were plotted, three different disappearance curves were obtained (fig. 1).

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The Amino Acid Requirements of the Pink Bollworm[1]

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ABSTRACT

The growth and development of the pink bollworm (*Pectinophora gossypiella* (Saund.)) on media containing various amino acids as a source of nitrogen is described. Optimum development was observed on a medium with an amino acid mixture based upon the composition of cottonseed protein. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine were found to be indispensable, but on a medium containing only these amino acids growth was slow and the adults were small. The inclusion of certain dispensable amino acids in the diets was necessary for optimum growth and development.

The growth and reproduction of the pink bollworm, *Pectinophora gossypiella* (Saund.), on sterile synthetic media has been described in previous papers (Vanderzant & Reiser 1956a, b; Vanderzant *et al.* 1957). These investigations showed that sucrose or glucose satisfied the carbohydrate requirements, that casein supplemented with cystine and glycine was an excellent dietary protein, and that cholesterol, choline, and linoleic acid were indispensable dietary constituents. Preliminary experiments also indicate that niacin, thiamine, pantothenic acid, folic acid, biotin, and probably vitamin B_{12} are essential for the normal development of the pink bollworm (Vanderzant 1957b).

Although excellent growth was observed on media with casein, these media cannot be used to determine the requirements for individual amino acids and their relationships to other substances. Furthermore, a medium with amino acids as a source of nitrogen would be desirable in the determination of the requirements for vitamins, minerals, and other trace nutrients that may be present in complex proteins such as casein.

Preliminary experiments with a medium in which casein was replaced with a mixture of known amino acids have recently been reported (Vanderzant 1957a). The present paper describes the effects of various amino acid mixtures and the omission of single amino acids from these mixtures.

GENERAL METHOD.—Except for changes in the amino acids (table 1), the composition of the media used in this study and the method of preparation were the same as that described in the previous paper. However, they differed from the original casein medium (Vanderzant & Reiser 1956b) in some of the other constituents. It was found that a portion of the Wesson's salts would not dissolve. A new salt mixture was developed which was easily suspended in the medium. Sucrose was replaced with glucose to reduce browning. Tween 80 was substituted for algianate as a stablizer. Cellulose was omitted because it has the tendency to settle out and clog the dispensing funnels. To reduce the osmotic pressure only about half as much carbodydrate and salts were used in the amino acid media as in the casein medium. These changes did not affect the rate of growth and development of the pink bollworm.

Newly hatched aseptic larvae were transferred aseptically to sterile rearing vials, one larva to each vial (Vanderzant & Reiser 1956a, b). They were kept in an incubator at 86° ± 1° F. and a relative humidity of 80 to 90%. The insects were observed at various intervals, and records made of their sex, date of pupation, pupal weights, emergence, and oviposition.

MIXTURES CONTAINING CASAMINO ACIDS.—In preliminary experiments casein was replaced with an equal quantity (5 grams per 100 grams of medium) of casamino acids. On this medium the larvae survived only a few days. Since tryptophan is destroyed by acid hydrolysis, from 25 to 100 mg. per 100 grams were added to this medium. In other media the amounts of casamino acids were reduced to 2.5 and 1.5 grams per 100 grams. Regardless of the concentrations of casamino acids and tryptophan, the larvae survived only 6 to 10 days, with the exception of two that became very small adults after 50 days (table 2). It is possible that (1) acid-hydrolyzed casein contains inhibitory principle(s) and/or (2) the levels and proportionality of the amino acids in the hydrolyzate are not satisfactory for the development of the pink bollworm.

AMINO ACID MIXTURES BASED UPON COMPOSITION OF CASEIN.—A mixture of amino acids based upon the composition of casein (House 1954) was tried as a replacement for casein, in the amount of 2 grams per 100 grams of medium. When greater amounts were tried, the limit of solubility of some of the amino acids was reached. Larvae placed on this medium died in a few days. This medium, however, contained several DL-amino acids, and some of

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[2] In cooperation with the Texas Agricultural Experiment Station, College Station.

Table 1.—Amino acid mixtures used in pink bollworm rearing media.[a]

AMINO ACID	No. 1	No. 2[b]	No. 3[b,c]	No. 4	No. 5[d]	No. 6[d]
Indispensable						
L-Arginine	0.076	—	—	0.100	0.140	0.311
L-Histidine hydrochloride	.038	—	—	.050	.070	.075
L-Isoleucine	.102	—	—	.188	.140	.113
L-Leucine	.296	—	—	.313	.234	.175
L-Lysine hydrochloride	.118	—	—	.150	.210	.113
L-Methionine	.060	—	—	.075	.075	.075
L-Phenylalanine	.078	—	—	.100	.100	.150
L-Threonine	.092	—	—	.113	.113	.100
L-Tryptophan	.024	—	—	.038	.053	.075
L-Valine	.104	—	—	.125	.094	.138
Dispensable						
L-Alanine	.038	.019	.119	.150	—	—
L-Aspartic acid	.082	.041	—	.100	—	—
L-Glutamic acid	.218	.109	—	.125	—	—
Glycine	.250	.125	—	.150	—	—
L-Proline	.168	.084	—	.050	—	—
L-Serine	.008	.004	—	.050	—	—
L-Cystine	.166	.083	—	.050	—	—
L-Tyrosine	.052	.026	—	.050	—	—
Total						
Indispensable	.988	.988	.988	1.252	1.229	1.325
Dispensable	.982	.491	.591	.725	.725	.725
Grand total	1.970	1.479	1.579	1.977	1.954	2.050

[a] Grams per 100 grams of medium.
[b] Indispensable amino acids same as in No. 1.
[c] Dispensable amino acids, except for alanine, same as in No. 2.
[d] Dispensable amino acids same as in No. 4.

Table 2.—Growth of the pink bollworm on media containing various amino acid mixtures.

Mixture[a]	Number of			Average Larval Period (Days)	Average Weight of Pupae (Milligrams)	
	Larvae	Pupae	Adults		Males	Females
Casamino acids plus L-tryptophan	43	2	2	52	—	—
No. 1[b]	162	0	—	—	—	—
No. 2	23	11	11	46		9.3 all
No. 3	22	18	18	31		10.7 all
No. 4	35	13	8	39	10.0	11.1
No. 5	25	23	18	30	12.6	16.4
No. 6[c]	120	110	107	24	15.2	18.9

[a] The composition of these mixtures is given in table 1.
[b] Total of several experiments in which other constituents of the medium were varied.
[c] Four experiments.

Table 3.—Effect of omission of amino acids indispensable for the rat on the development of the pink bollworm.

Amino Acid Omitted	Total Number of Larvae	Larvae Alive on 14th Day	
		Number	Instar
L-Arginine	47	0	1
L-Histidine	46	2	1
L-Isoleucine	52	4	2
L-Leucine	57	3	2
L-Lysine	40	7	2
L-Methionine	44	7	1
L-Phenylalanine	58	1	1
L-Threonine	53	7	1
L-Tryptophan	41	3	2
L-Valine	46	8	2

the D-amino acids may have toxic or antagonistic properties. In subsequent experiments only L-amino acids were used.

The results with other mixtures of amino acids are presented in table 2 and their compositions in table 1. The amino acids were divided into two groups, dispensable and indispensable, as determined for the diet of the rat (Rose 1938).

Mixture No. 1 was based upon the composition of casein with supplementary amounts of glycine and cystine. This medium failed to support growth of the pink bollworm. In subsequent experiments several media with the same amino acid composition but with increased amounts (50 and 100%) of carbohydrate, vitamins, or salts were tried, but no larvae developed beyond the second instar (table 2).

Several reports (Goldberg & de Meillon 1948, McGinnis *et al.* 1956) have appeared that glutamic acid and/or aspartic acid at high concentrations inhibit growth of certain insects. For this reason only half the amounts of the dispensable amino acids were used in mixture No. 2. Eleven of the 23 larvae became adults. The average larval period was 46 days, and the average pupal weight 9.3 mg.

In subsequent experiments the concentration of a single dispensable amino acid was increased in a series of eight mixtures. Except for the amino acid tested, the composition of these mixtures was the same as for No. 2. Alanine, aspartic acid, glutamic acid, and glycine were increased by 0.1 gram and proline, serine, cystine, and tyrosine by 0.05 gram. In general, the growth was similar for all mixtures and data for a mixture with more alanine (No. 3) are given as an example. More adults developed than on medium No. 2, and the average larval period was reduced by 15 days. Some growth stimulation may have resulted from the the increased amino acid content or from a balance between the amino acids rather than the specific need for any one. The slight stimulation observed with added glutamic acid seems to indicate that, within the limits used in this experiment, it did not exhibit toxic properties.

In mixture No. 4 the total amount of amino acids was adjusted again to approximately 2 grams. The indispensable amino acids comprised about two-thirds of the total and were still based on casein composition. The best average values for the dispensable amino acids, as determined in the previous experiments, were used. Growth on this medium was slow, and only a small percentage of the larvae developed into adults.

In mixture No. 5 changes were made in the content of the indispensable amino acids. Cottonseed protein contains more of the basic amino acids and less leucine and isoleucine than casein. Therefore, the amounts of arginine, histidine, lysine, and tryptophan were increased and those of leucine, isoleucine, and valine decreased. The dispensable amino acids were the same as in mixture No. 4. Pink bollworms reared on this medium were larger than those reared on previous media (table 2).

Mixture No. 6 was based upon the composition of cottonseed protein with respect to the indispensable amino acids (Lyman *et al.* 1947). On this medium the average larval period was about 4 days longer than on the basal casein medium, probably owing to an initial lag in growth. In the early phase of their development the larvae did not feed so readily on the jelly-like amino acid medium as they did on the firmer casein medium. Adults reared on this medium deposited fertile eggs, from which a second generation of adults was reared.

Omission of Individual Amino Acids.—To test the amino acid requirements of the pink bollworm, those acids indispensable for the rat were omitted one at a time from mixture No. 6 (table 3). With the exception of arginine, which was replaced with an equal quantity of glycine, the total amino acid content was reduced slightly each time. On each of the deficient media most of the larvae were dead by the 14th day, when larvae reared on a complete medium were already in the fourth instar. Observations were made as long as any larvae lived, but none developed beyond the second instar.

To check these media for possible errors in preparation, a few drops of a sterile solution of the amino acid omitted from the diet were added to several rearing vials with the deficient medium and, after the solutions were absorbed, larvae were transferred to these vials. In each case the larvae became adults. The data indicate that all these amino acids are indispensable for the growth and development of the pink bollworm.

On a medium containing only these 10 indispensable amino acids as sources of nitrogen at 2 grams per 100 grams, only 50% of the larvae became adults, with an average pupal weight of 9.2 mg. (table 4). Previously, when a mixture of indispensable amino acids (No. 1) was

Table 4.—Effect of omission of certain amino acids on the development of the pink bollworm.

Modification of Basal Medium	Number of				Average Weight of Pupae (Milligrams)	
	Live Larvae	Dead Larvae	Pupae	Adults	Males	Females
None	27	3	24	24	17.0	20.1
Indispensable only, 2 grams	36	18	18	18	9.2 all	
L-Cysteine hydrochloride substituted for L-cystine	48	5	43	42	18.6	21.0
Omission of						
L-Cystine	42	7	35	33	14.8	18.9
L-Tyrosine	53	3	50	50	15.4	17.9
Glycine	36	3	33	33	15.1	22.9
L-Alanine	24	2	22	20	17.1	21.6

tested similarly, no larvae grew beyond the second instar.

The single omission of cystine, tyrosine, glycine, or alanine from the medium had no apparent detrimental effect (table 4). Furthermore, cysteine could be substituted for cystine with similar results. The pupal weights were within the normal range. Eggs were obtained from moths reared on these media, but no data on the number of eggs per female were obtained.

Preliminary experiments indicate that the omission of glutamic acid, aspartic acid, proline, or serine from the medium does not have a significant effect on the development of the pink bollworm.

DISCUSSION.—Several investigators have used successfully amino acid mixtures based upon the composition of casein in insect diets, but on most diets growth was not as good as with natural diets or those containing casein. It has been shown that hydrolyzed casein or mixtures of amino acids based on the composition of casein do not replace it completely in the diets of many animals (Almquist 1951). This observation has been verified in the present study with the pink bollworm. Further studies with different amino acid mixtures indicated that there was probably an improper balance of amino acids in the preliminary mixtures. Elvehjem (1956) has reviewed the studies of amino acid imbalance in animal nutrition, which show that the toxicity and antagonisms of natural amino acids complicate the determination of nutritional requirements.

Investigations of the dietary amino acids needed for insects have shown a striking similarity to those needed by higher animals. The pink bollworm has now been added to the list of insects (Moore 1946, Lemonde & Bernard 1951, Fraenkel & Printy 1954, House 1954, Ishii & Hirano 1955) that have amino acid requirements similar to those of the rat.

The requirement for sulfur-containing amino acids apparently can be furnished by methionine, because cystine is not needed in an otherwise complete medium. At low dietary levels of methionine, cystine probably would have a sparing effect. Hodgson *et al.* (1956) have shown that the black blow fly (*Phormia regina* (Meig.)) needs either methionine or cystine.

The amount of phenylalanine in the medium was also sufficient to supply the tyrosine requirements, because adults developed when tyrosine was omitted. No requirement was found for glycine, although it has been reported to be essential for certain insects (Goldberg & de Meillon 1948, Hinton *et al.* 1951, Davis 1956).

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Resistance of the Spotted Alfalfa Aphid to Certain Organophosphorus Insecticides in Southern California[1]

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ABSTRACT

During the late summer of 1956 an increasing number of failures were reported in alfalfa fields where parathion and malathion were used to combat the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in southern California. A survey of the troubled area strongly indicated the presence of a resistant strain of aphids. Field tests showed that the aphid has developed a low degree of resistance to parathion and Trithion in localized areas. In one test, malathion, Phosdrin, and demeton (Systox) applied at 9.1, 0.7, and 0.9 ounces per acre, respectively, gave only mediocre control. These same dosages gave excellent control of susceptible aphid populations. Laboratory studies showed that the aphid had developed approximately 4-fold resistance to parathion residues. If time of exposure to mortality is considered, the degree of resistance is probably even greater. Resistant aphids continued reproduction when exposed to the toxicant, and many of their young survived. Reproduction in nonresistant aphids was markedly reduced, and the young died. The developmental pattern of resistance in parthenogenetic species does not progress in the same manner as in multivoltine bisexual species. Resistance probably arose through mutation after the aphid arrived in North America.

During the late summer of 1956 an increasing number of failures were reported in fields where parathion and malathion were used to control the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in southern California. Of the two materials, parathion has been used more frequently as an aphid control measure in southern California and has been mentioned more often where control has been unsatisfactory.

Since excellent control of the spotted alfalfa aphid has been obtained with a number of organophosphorus and chlorinated hydrocarbon insecticides (Tuttle & Butler 1954, Reynolds & Anderson 1955, Tuttle 1956, Stern & Reynolds 1957), it was first thought that growers were not using the recommended chemical dosage, that methods of application were not satisfactory, or that, possibly, heavy alate aphid invasions were responsible for a rapid reinfestation following treatment. However, investigations of several failures showed that spray equipment and methods of application were satisfactory and that the numbers of alate aphids on the alfalfa plants in the fields were not unusually large. Furthermore, growers stated that control was mediocre even when the recommended dosage of parathion (2 to 3 ounces per acre, applied by ground equipment) was doubled. In a survey of these heavily treated fields, large numbers of older nymphs and apterous adults were found to have survived the insecticide applications.

State officials took samples of parathion from containers on farms where treatments had failed, and these samples were sent to the California State Bureau of Chemistry for analysis. These analyses showed that the percentage of active ingredient in the samples complied with State and Federal labeling regulations. Thus there appeared to be a good possibility that the spotted alfalfa aphid had developed some degree of resistance to parathion and possibly to other organophosphorus insecticides.

There have been few authenticated cases of the development of resistance to insecticides by aphid species. Knipling (1954) in a discussion of the insecticide resistance problem, observes that parathion- and DDT-resistant strains of the potato aphid, *Macrosiphum solanifolii* (Ashm.), and of the green peach aphid, *Myzus persicae* (Sulzer), have appeared on fruits and vegetables. Anthon (1955) failed to obtain satisfactory control of the green peach aphid, *M. persicae*, when using parathion and malathion in field experiments. Apparently, TEPP was also inadequate in two cases, though excellent control was obtained on laboratory-reared aphids. Commercial pest control applicators and entomologists in the Salinas Valley and parts of southern California have also reported failures in green peach aphid control. Entomologists in the Cotton Belt have mentioned that the cotton aphid, *Aphis gossypii* Glover, has occasionally shown signs of being resistant to insecticides. However, except in the report on fumigation tests by Boyce (1928), detailed reports of resistance in the cotton aphid have not appeared in the literature.

In 1954, Michelbacher *et al.* reported that a strain of the walnut aphid, *Chromaphis juglandicola* (Kltb.), had developed a 7-fold resistance to parathion near San Jose, Calif. This aphid is apparently becoming increasingly difficult to control in certain walnut areas of southern California. Since 1950 it has been necessary to increase the recommended dosage of parathion in order for growers to obtain consistently good results. In 1950 the recommended dosage for control of the walnut aphid was 1 pound of 25% parathion wettable powder per 100 gallons of water, and by 1957, 4 to 6 pounds of 25% parathion wettable powder per 100 gallons of water were recommended for walnut aphid control (Ortega 1950, 1957).

If the data obtained by Randolph (1957) on the spotted alfalfa aphid in Texas in 1955 are compared with the data obtained there in 1956, it seems possible that the preliminary indications of resistance are appearing in Texas much as encountered in California. Additional studies will have to be made, however, before it can be concluded that localized aphid populations in Texas are resistant to organophosphorus insecticides.

FIELD EXPERIMENTS AND RESULTS.—All materials used in the field tests were applied as sprays. The method of evaluating each compound was the same in all tests. Single alfalfa stems were cut just above ground level, and the living apterous aphids on them were counted. In each experiment treatments were replicated four or

[1] Paper No. 1010, University of California Citrus Experiment Station, Riverside, California. Accepted for publication November 25, 1957.

Table 1.—Effectiveness of various insecticide sprays applied by ground equipment on resistant populations of the spotted alfalfa aphid in plots near Hinkley, California.

Material	Gallons per Acre	Ounces Active Ingredient per Acre	Interval Between Treatment and Sampling (Days)					
			First Sample		Second Sample		Third Sample	
			No. Apterous Aphids per 100 Stems[a]	Per Cent Reduction from Check	No. Apterous Aphids per 100 Stems[a]	Per Cent Reduction from Check	No. Apterous Aphids per 100 Stems[a]	Per Cent Reduction from Check
Test No. 1.—Sprays Applied September 12, 1956								
			1 Day		2 Days			
Parathion	9.5	2.3	7,159	77	4,957	86		
Trithion	15.0	3.8	5,041	84	2,948	91		
Phosdrin	10.5	0.7	368	99	166	99		
Phosdrin	10.5	1.3	112	99	26	99		
Check	—	—	30,890	—	34,095	—		
Test No. 2.—Sprays Applied November 1, 1956								
			1 Day		2 Days		6 Days	
Parathion	15.7	5.3	4,439	47	2,510	73	1,004	92
Parathion	11.5	5.7	3,483	59	1,700	82	328	97
Parathion	12.1	8.1	2,739	68	1,270	86	115	99
Malathion	12.7	12.7	2,091	75	320	98	42	99
Demeton	11.5	3.9	52	99	21	99	49	99
Check	—	—	8,446	—	9,204	—	12,023	—
Test No. 3.—Sprays Applied March 28, 1957								
			1 Day		4 Days			
Parathion	12.0	4.0	500	73	218	87		
Trithion	12.0	12.0	651	64	194	88		
Malathion	11.4	9.5	18	98	35	98		
Phosdrin	12.0	1.0	1	99	3	99		
Demeton	11.4	0.7	32	98	109	93		
Demeton	12.0	1.5	1	99	8	99		
Check	—	—	1,822	—	1,664	—		

[a] In test No. 2 five replicates were made and 125 stems were sampled per treatment.

five times. Twenty-five alfalfa stems were counted in each replicate to give 100 stems per treatment where four replicates were used and 125 stems where five were used. Effectiveness of a treatment was determined by the reduction of aphids as compared with the untreated check. In all tests a ground sprayer was used, equipped with a 30-foot boom containing No. 4X hollow-cone nozzles arranged 18 inches apart for broadcast spraying. All sprays were applied at a pressure of 60 pounds per square inch.

Test No. 1.—On September 12, 1956, sprays were applied on alfalfa in plots near Hinkley, Calif., in the Mojave Desert. The alfalfa was about 15 inches tall. Each treatment was replicated four times, and each replicate plot was 60 feet wide and 100 feet long. The spray materials were evaluated 1 and 2 days after application. On the third day after application the grower re-treated the entire field, thus preventing further evaluation of the spray materials. The data are summarized in table 1.

When 2 to 3 ounces of parathion were applied per acre on a nonresistant aphid population, control was very effective 24 hours after application. Nearly all the aphids dropped from the plants a few hours after treatment (Stern & Reynolds 1957). In this test parathion applied at 2.3 ounces per acre gave 77% control 24 hours after application. Two days after treatment control increased to 86%. Trithion applied at 3.8 ounces per acre failed to give satisfactory control. Phosdrin was applied at 0.7 and 1.3 ounces per acre, and both dosages gave effective control 24 hours after treatment. Results with parathion were comparable to those obtained by growers using a similar dosage. At the time of testing, a few growers at Hinkley were using 4 to 6 ounces of parathion per acre. Observations of these grower treatments showed that initial results were inadequate. Control increased a few days later, but satisfactory control was not obtained and the adults surviving treatment, plus a few immigrating alate aphids, soon produced a sufficient number of young to warrant another spray application.

Test No. 2.—On November 1, 1956, another test was conducted in the same area (near Hinkley). The alfalfa was about 8 inches tall. The sprays were applied in the same manner as in test 1. Each treatment was replicated five times, and each replicate plot was 30 feet wide and 100 feet long. The spray materials were evaluated 1, 2, and 6 days after application. The data are summarized in table 1.

Parathion was applied at three dosages, 5.3, 5.7, and 8.1 ounces per acre. All gave very poor control 24 hours after application. Two days after treatment, control had improved slightly but was still below a satisfactory level. Six days after treatment there was considerable improvement in those plots treated with 5.7 or 8.1 ounces of parathion. Where 5.3 ounces were used, an

Table 2.—Effectiveness of various insecticide sprays applied April 10, 1957 by ground equipment on a resistant population of the spotted alfalfa aphid at Thermal, California (test No. 4).

Material	Gallons per Acre	Ounces Active Ingredient per Acre	Interval Between Treatment and Sampling: 2 Days — No. Apterous Aphids per 100 Stems	2 Days — Per Cent Reduction from Check	5 Days — No. Apterous Aphids per 100 Stems	5 Days — Per Cent Reduction from Check	8 Days — No. Apterous Aphids per 100 Stems	8 Days — Per Cent Reduction from Check
Parathion	12.1	4.0	1,050	80	1,874	75	2,608	64
Trithion	10.9	7.3	1,677	68	3,566	54	3,190	56
Malathion	10.9	9.1	638	88	1,387	82	2,589	64
Phosdrin	11.7	0.7	630	88	1,272	84	1,168	84
Demeton	11.1	0.5	1,693	68	2,620	66	1,746	76
Demeton	10.9	0.9	1,426	73	1,678	78	1,141	84
Check	—	—	5,268	—	7,793	—	7,280	—

average of eight mature or nearly mature aphids remained per stem. During warmer periods this number of aphids could rapidly increase to such a potentially damaging population that re-treatment would be necessary. Demeton applied at 3.9 ounces per acre gave very effective control 24 hours after treatment. Malathion applied at 12.7 ounces per acre gave unsatisfactory control 24 hours after treatment, but effective control within 48 hours after treatment.

In this test there was delayed mortality where parathion was used. Delayed mortality was also noted at Hinkley in fields which growers had treated with parathion. This occurrence is exemplified by a poor initial kill followed later by incomplete control where a low dosage was used and by poor initial control followed by effective cleanup as the dosage was increased. In contrast, at Thermal (see table 2), parathion, malathion, and Trithion gave poor initial control which gradually decreased in relation to the check as the surviving adults continued to reproduce.

Test No. 3.—On March 28, 1957, a third test was conducted in the Hinkley area. The alfalfa was about 15 inches tall. The sprays were applied as in previous tests. Each treatment was replicated four times, and each replicate plot was 30 feet wide and 325 feet long. The spray materials were evaluated 1 and 4 days after application. The data are summarized in table 1.

Demeton was applied at two dosages, 0.7 and 1.5 ounces per acre. Both concentrations gave satisfactory control 24 hours after application. Where the lighter dosage was applied the survivors were mostly mature aphids. Four days after application these survivors had produced a number of young, and populations increased slightly. Phosdrin and malathion gave satisfactory control during the testing period. Parathion and Trithion were used at dosages above the concentration required for control of nonresistant aphids and failed to give satisfactory control.

During 1955 and 1956, Trithion was used in a number of tests and was applied at 4.5 ounces or more per acre. In these tests, conducted on non-resistant aphid populations, control was satisfactory 24 hours after application (Stern & Reynolds 1958). Against resistant aphids, 3.8 ounces of Trithion per acre failed to give adequate control in test No. 1, and 12.0 ounces per acre failed to give adequate control in test No. 3.

Test No. 4.—On April 10, 1957, spray materials were applied on alfalfa at Thermal, California. The alfalfa was about 12 inches tall and the materials were applied in the same manner as in the first three tests. Each treatment was replicated four times, and each replicate plot was 30 feet wide and 300 feet long. The spray materials were evaluated 2, 5, and 8 days after application. The data are summarized in table 2.

This was a new field of alfalfa planted in October, 1956. When the plants were in the cotyledon stage a potentially damaging aphid population developed and the field was treated with parathion. Eight additional parathion treatments were applied during the winter months, the last treatment being in early March. Thus the aphid population in this field at the time of testing was probably composed of a high percentage of aphids with varying degrees of resistance. The remaining portion of the population consisted of the offspring of alate aphids entering the field from adjacent areas. These aphids may or may not have been resistant to organophosphorus insecticides.

All materials failed to give satisfactory control. Phosdrin and malathion were applied at dosage levels which had given adequate control of susceptible aphids, while parathion and Trithion were applied in excess of that required to control susceptible aphids. Demeton was applied at two dosages, 0.5 and 0.9 ounce per acre. When demeton was applied by ground equipment at 0.75 to 1 ounce per acre, on alfalfa bearing nonresistant aphids, control was adequate 24 hours after application (unpublished data of V. M. Stern and R. van den Bosch). Control improved in the plots sprayed with demeton because large numbers of lady beetles survived the treatment and continued to feed on the surviving resistant aphids (unpublished data of V. M. Stern and R. van den Bosch).

Two other tests were conducted in California during the winter of 1956. In one test, at Lancaster, parathion applied at 8 ounces per acre failed to give effective control of aphids 6 days after application. In a second test, at Thermal, parathion applied at 4.4 ounces per acre gave 15% control 24 hours after treatment, and 77% control 8 days after application.

LABORATORY TECHNIQUE AND RESULTS.—When it was known that 2 to 3 ounces of parathion no longer gave adequate aphid control in certain areas, aphid samples

were brought into the laboratory for study. In sampling a parthenogenetic insect it should be recognized that a given population may be composed of one or hundreds of distinct clones. This population may or may not show gradating characters as in bisexual insects. Thus a character or physiological process may be present in a variable frequency in a localized population. The frequency will depend on long- or short-term, widespread or restricted selective pressures, or on the migration of new individuals into the area. If parthenogenetic aphids are sampled in a resistant-aphid area after an insecticide application, a short-term, restricted selective pressure, many of the surviving individuals will be resistant. Through clonal development, resistance in the treated field will be maintained, at least for a certain period, if the surviving resistant individuals are not inferior in the absence of the insecticide, and if large numbers of susceptible individuals do not move into the treated area.

For use as a nonresistant population, spotted alfalfa aphids were collected at Big Pine, Calif., in the Owens Valley, which lies along the eastern slope of the Sierra Nevada Mountains. This area was selected as a source of nonresistant aphids because field tests showed that 2 to 3 ounces of parathion still gave excellent aphid control 24 hours after application (Stern & Reynolds 1957). In addition, the aphids had only recently increased to damaging numbers in the area, and the extent of chemical control on alfalfa in the Owens Valley was negligible in comparison with that in other areas in southern California. Resistant aphid stocks were collected near Hinkley, Calif., where, as indicated above, 2 to 3 ounces of parathion no longer gave adequate control.

Aphids brought into the laboratory were exposed to various concentrations of parathion by the residue-dip method: small alfalfa bouquets were dipped in various concentrations of parathion, and aphids were then placed on the bouquets. In preparing a standard solution, 25 grams of technical parathion was used, and xylene was added to increase the solution to 100 ml. As each concentration of parathion was prepared for the tests, a constant percentage of emulsifying agent (Triton X-100) was added. In this study the emulsifying agent amounted to 1/1000% of each solution. The parathion solutions were well emulsified in a Waring Blendor before the alfalfa bouquets were dipped in them. In preparing the bouquets the top 3 inches of 2 or 3 alfalfa stems were wrapped in cotton and placed snugly in small vials filled with water. The bouquets were then dipped for 10 seconds in the desired concentration of parathion, taken out, and shaken to remove the excess liquid. After the alfalfa stems had dried, single bouquets were placed in ½-pint cardboard ice-cream cartons. Fifty mature apterous aphids were used to determine the percentage mortality for each concentration of parathion. In the work with resistant aphids, this procedure was repeated on three occasions for each concentration of parathion. The three resultant mortality percentages were averaged and used for plotting the line in figure 1. In the work with nonresistant aphids the procedures were repeated twice, the results were averaged and this figure used for plotting the line in figure 1.

The 50 mature aphids used for each concentration of parathion were divided into three lots, 17 aphids being placed on each of two bouquets in two of the ice-cream cartons, and 16 on the third. Cotton gauze was used as a cover for each carton, and a rubber band held the gauze in place to prevent escape of the aphids. Forty-eight hours after the aphids had been placed on the bouquets the percentage mortality was recorded. Checks of 50 mature aphids were used for each test. Mortality of the untreated aphids was negligible. The results are shown in figure 1.

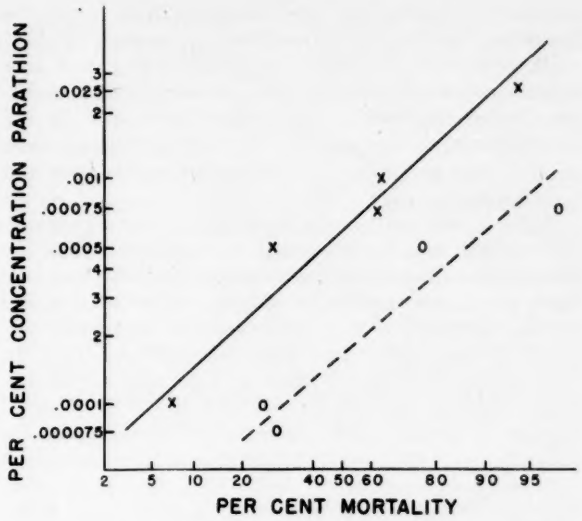

FIG. 1.—Dosage-mortality curves showing the effect of various dosages of parathion on mortality of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in the laboratory: resistant strain (solid line) and nonresistant strain (broken line).

Results of laboratory tests show that aphids from Hinkley, developed approximately 4-fold resistance to parathion by late 1956. The LC-50 of the Hinkley (resistant) strain was approximately 0.00059% parathion, while the LC-50 of the Big Pine (nonresistant) strain was approximately 0.00015% parathion. The LC-90 of the Hinkley strain was approximately 0.0024%, while the LC-90 of the Big Pine aphids was approximately 0.00052% parathion. Actually, the degree of resistance separating the two populations is greater than 4-fold if the time from exposure to mortality is considered. A 48-hour exposure period was arbitrarily selected. By allowing 48 hours to elapse, the nonresistant aphids were favored, particularly at the higher dosages, since a great percentage of them died within 24 hours. At the same dosage, resistant aphids required a longer period for mortality.

It should also be noted that the resistant aphids continued to reproduce in the laboratory at parathion concentrations of 0.001% and below, and many of their young survived. Thus where resistant aphids were being tested there was often an increase in numbers above those of the initial samples. Reproduction in nonresistant aphids was markedly reduced, and the young died. Only the surviving adults were counted in each test.

DISCUSSION.—The development of resistance to insecticides by totally parthenogenetic insects does not progress in the same pattern as with bisexual insects. In bisexual species, continued selection with the insecticide,

followed by segregation and recombination, allow the frequency and level of resistance to increase. Where multiple factors are involved, selection, segregation, and recombination allow a continuing development of resistance which may end in super-resistant strains. In the development of resistance by a totally parthenogenetic species, segregation and recombination are lacking and only selection occurs.

Theoretically, with clonal development, resistance cannot increase in a species except by mutation. When the species is parthenogenetic over most of the year, but may have one sexual generation a year, as in some aphid species, segregation and recombination may occur annually or may occur only under special conditions. In such instances the development of resistance is probably more nearly comparable to that of a univoltine bisexual species. In totally parthenogenetic species resistance apparently develops slowly, and additional time is required to develop super-resistant strains by a pattern of selection, parthenogenetic reproduction, mutation, and selection, as compared with multivoltine bisexual species in which segregation and recombination occur in each generation.

Resistance in the spotted alfalfa aphid is thought to have developed by mutation after the aphid arrived in North America. According to Dickson *et al.* (1955), the aphid may have been carried from the Old World to central New Mexico by airplane during the summer or fall of 1953. Establishment may have been made by a very limited number of aphids or, conceivably, by a single individual.

It is not known whether the gene pool of the parent population was supplemented by later arrivals from the Old World. Even so, in analyzing possibilities, it would appear that though the aphid is now widely distributed and has a tremendous population density, its original gene pool in North America was limited. It is thought that the factor or factors controlling resistance to organophosphorus insecticides were not present in the original gene pool and that resistance arose through one or more mutations after arrival. Reasons for this hypothesis are that resistance is restricted to localized populations in North America. Had the resistance factor been well distributed in Old World populations of this aphid and thus introduced into this country, numerous chemical control failures would have occurred in 1954 and 1955 when thousands of acres of alfalfa were being treated. Since this aphid appears to be totally parthenogenetic (Dickson *et al.* 1958), clonal development would have permitted widespread resistance to occur almost immediately after the first few chemical control measures were undertaken.

If the factor for resistance existed in low frequency in the Old World there would appear to be an infinitely small possibility that one of these carriers was among the small group or was the single individual that first reached North America. If the factor or factors for resistance had been carried by the original invaders it would also be expected that resistance would have become a problem in 1954 or certainly by 1955. However, field tests show that resistance is localized and did not appear in California until late summer of 1956.

Randolph's (1957) data, when compared with results reported in the present paper, appear to show signs of resistance in the aphid population near College Station, Texas. Within a short time, should that population prove to be resistant, it would seem that resistance there developed independently of that in California, since the two areas are separated by hundreds of miles, and between the two points there are many alfalfa-growing areas where resistant aphid populations have yet to occur.

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Effect of a California Spray Oil on Transpiration of Citrus[1]

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Abstract

The effect on transpiration in plants of several varieties of citrus, of a representative California foliage spray oil applied according to present-day practice for control of citrus pests in the field was investigated in laboratory experiments. Plants of Eureka lemon, Bearss lime, navel orange, and Valencia orange were grown in pots in the glasshouse; temperature control permitted a maximum of 92° F. during the day and a minimum of 66° F. during the night. Transpiration was measured by determining the loss in total weight of the potted plant during a period of 7 hours from morning to afternoon; the surface area of the foliage was measured and transpiration was expressed as milligrams of water transpired per square centimeter of leaf surface per hour. On the first day after the application of 1.75% spray oil in aqueous mixture, transpiration of the oil-sprayed plants was reduced to one-third that of untreated plants. Oil-sprayed plants recovered with time, and analyses showed that the correlation with the log 10 of time is highly significant. The available evidence indicates that reduction in transpiration in citrus foliage after the application of oil spray is due to physical interference by the spray oil on or in the leaf tissue and that recovery of transpiration occurs with dissipation of oil from the leaves. Transpiration was restored to original levels in the oil-sprayed plants in 3 to 5 weeks after the spray was applied.

Experiments conducted by Knight *et al.* (1929) demonstrated that application of petroleum oil caused a sharp drop in the transpiration rate of citrus. The oils used were either lower- or higher-boiling fractions than the spray oils commonly used now in citrus pest control in southern California. Only one of the lighter oils was applied in aqueous spray mixture; the other oils were painted on the leaves, and the amount of oil present was considerably more than that deposited by the conventional oil-spray mixtures used today. Restoration of transpiration in 1 to 3 weeks after application of the light oils, and in 6 to 7 weeks after application of the heavy oil, was reported. Bartholomew (1936) reported observations indicating that the depressing effect of spray oil on transpiration of citrus is only temporary.

The experiments reported here were conducted to investigate the effect of a representative California foliage spray oil, used according to present-day practice for control of citrus pests in the field, on transpiration in plants of several varieties of citrus.

Materials and Methods.—The plants used were obtained by propagating rooted stem cuttings of Eureka lemon, Valencia orange, navel orange, and Bearss lime. The varieties are listed here in the order of increasing susceptibility to leaf drop following oil-spray applications in the field in southern California (Ebeling 1950). Since Bearss lime is much less tolerant than Eureka lemon to oil spray in the field, comparisons of effects on these two varieties were of particular interest.

Suitable terminals from the peripheral foliage of orchard trees which had never been treated with oil spray or other chemical pesticides were collected and brought into the laboratory. Stem cuttings 6 inches long, with three leaves at the upper end of each, were taken. The lower end of the stem was cut transversely, and three small notches were made in the bark, equally spaced around the circumference of the stem. During collection and preparation the foliage and cuttings were kept in water as much of the time as possible.

The lower end of each stem cutting was dipped in talcum powder impregnated with 0.2% *alpha*-naphthaleneacetic acid to induce rooting, and was placed in a covered rooting bed of washed sand. The rooting beds were inside a steam-heated glasshouse operated at a minimum temperature of 70° F., so bottom heat was not used in the sand bed. A clock-operated spraying system was used to spray the cuttings with water intermittently as needed to keep the leaves moist. The cuttings were left in the sand until a minimum number of two roots, each 3 inches in length or longer, had developed. Under our experimental conditions an interval of 4 to 5 weeks was required for lemon or lime cuttings to root, but more than 90% of the cuttings of these varieties developed roots readily. Stem cuttings of peripheral foliage of Valencia and navel orange trees did not root as readily as those of lemon; they required as long as 12 weeks in the sand bed, and only ⅓ to ½ of the cuttings developed roots. *Alpha*-naphthaleneacetic acid was the best of several rooting compounds tried.

The rooted cuttings were transplanted to a mixture of soil, sand, and peat (2:1:1) in 6-inch pots and placed in a glasshouse room in which temperature control permitted a maximum of 92° F. during the day and a minimum of 66° F. at night. Transmission of sunlight through the roof and walls of the glasshouse was restricted only by the minimum deposit of whiting required to maintain the maximum temperature. Fertilizer was supplied by application of full-strength Hoagland's solution each month. The plants were held in the glasshouse until the foliage reached a height of 18 to 24 inches. Three weeks before application of the oil spray all the foliage younger than fully expanded leaves was removed from each plant; thereafter, until the close of the experiment, the plants were inspected daily and new growth was removed as it appeared. Prior to treatment, plants of each variety were arranged in pairs. Selection was made after pretreatment transpiration determinations so that members of each pair were as comparable as possible with respect to rate of transpiration, height and amount of foliage.

The oil used was a California medium-grade foliage spray oil (Erb 1932, Marshall 1932) having the following selected properties: minimum unsulfonated residue, 92.5; composition by weight: 1.6% aromatic carbon, 51.4% naphthenic carbon, and 47.0% paraffinic carbon; fractional distillation: 10% at 590° F., and 90% at 687° F. The oil was formulated as an emulsive-type spray oil by the addition of 13 ml. of a non-ionic, oil-soluble

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Table 1.—Inhibition of transpiration in citrus plants at various intervals after application of 1.75% medium-grade California emulsive spray oil in aqueous mixture.[a]

Days After Treatment	Eureka Lemon		Bearss Lime		Navel Orange		Valencia Orange	
	No. of Pairs	Mean Difference $\pm$ s.e.$_{\bar{d}}$	No. of Pairs	Mean Difference $\pm$ s.e.$_{\bar{d}}$	No. of Pairs	Mean Difference $\pm$ s.e.$_{\bar{d}}$	No. of Pairs	Mean Difference $\pm$ s.e.$_{\bar{d}}$
0[b]	10	1.2±1.0	10	−1.0±0.8	9	0.8±0.9	4	−0.2±1.9
1	10	8.2±0.7	10	4.9±0.9	9	5.7±0.8	4	5.7±0.8
3	10	2.9±0.5	10	1.2±0.3	9	4.4±0.7	4	2.9±0.4
7	10	1.8±0.6	10	1.1±0.9	9	3.5±0.6	4	2.8±0.6
10	10	1.2±0.4	10	0.9±0.9	9	1.4±0.5	4	0.9±0.5
14	10	1.3±0.9	10	−0.2±0.8	9	2.0±0.8	4	2.2±0.5
17	10	1.5±0.7	10	0.2±0.8	9	1.4±0.8	4	1.4±0.6
23	10	1.1±1.0	10	−0.3±0.9	9	−0.2±0.9	4	0.6±0.8
30	10	0.6±0.7	10	0.2±0.7	9	−0.9±0.6	2	−1.2±0.8
37	10	0.8±0.7	10	1.0±0.5	9	−0.2±0.3	3	−0.3±0.8
44	10	1.0±1.0	10	0.6±0.8	9	0.2±0.9	3	−0.4±1.6

[a] Mean difference, in milligrams of water transpired per square centimeter of leaf surface per hour, between paired untreated and sprayed plants, together with standard error of mean difference.
[b] Pretreatment.

emulsifier mixture (2 parts No. 1159 and 1 part No. A 023 emulsifiers; E. F. Houghton and Co., Philadelphia Pa.) per gallon of oil.

A concentration of 1.75% of the spray oil in aqueous spray mixture was applied at a spray pressure of 500 pounds per square inch by means of a conventional orchard spray rig utilizing a reciprocating-type pressure pump. The foliage of the plant was placed in the spray stream and moved about until it was wet thoroughly. From each pair of plants, one plant taken at random was sprayed with oil, and the other was left untreated.

The transpiration of each plant was measured on designated days by determining the loss in total weight of the plant to 0.1 gram during an interval of about 7 hours between morning and afternoon. The elapsed time in minutes was recorded for each plant for each transpiration measurement. Before the plant was weighed in the morning, the pot in which it was planted was placed in a watertight polyethylene freezer bag which was gathered at the top, around the trunk, leaving the foliage of the plant exposed. A cork gasket carefully shaped to the trunk to ensure a tight fit was placed around the trunk to protect the bark tissue from direct contact with the polyethylene material. The halves of the cork gasket were bound together with masking tape, and the top of the bag was closed against the gasket and tied tightly with a cord. After the plant had been weighed in the afternoon, the bag was opened and water was added to the soil in the pot, as needed. Leaves dropped by the plants were checked daily. Each leaf dropped was identified with the plant from which it came, and a record was made of the date and of the surface area of the leaf at the time it was found. At the end of the series of transpiration measurements, the leaves of each plant were picked and their total surface area was determined by means of a photoelectric arealimeter (Gunther 1948). Transpiration for each determination was expressed in milligrams of water transpired per square centimeter of leaf surface per hour.

Discussion of Results.—The rate of transpiration of the oil-sprayed plant was subtracted from that of the untreated plant for each pair of plants for each measurement of transpiration. The means of the differences and the respective standard errors of the mean differences are listed in table 1. The data for the first day after treatment show that a considerable reduction in transpiration occurred in the oil-sprayed leaves immediately after the spraying. The data for successive days show that the effect of the oil is temporary and that recovery of transpiration occurs with time. Curves plotted for various expressions of transpiration vs. time indicated a strong negative curvilinear association. Various transformations for time were tried in correlation calculations. Of these, an excellent expression of correlation was found for the log 10 of time vs. the mean difference in milligrams of water transpired per square centimeter per hour. Calculated data for correlations of log 10 of time in hours vs. water transpired are given in table 2. The coefficient of X in the equations is b, the regression coefficient or slope of the line; since the correlation of time vs. transpiration is negative, both b and r have a minus sign.

Inspection of the data of table 1 indicates that most of the recovery of transpiration in the oil-sprayed plants occurs within the first 3 weeks after spraying. Differences in water transpired on the 30th day after spraying and thereafter, appear to vary at a base level equivalent to that found in the pretreatment determination; therefore, the decision was taken to use the data for each determination of transpiration through the 23rd day after spraying for the calculations of correlations of time vs. transpiration, as shown in table 2.

Table 2.—Calculated data for correlation of inhibition of transpiration vs. time (data from table 1) and the derived expected time in days for recovery of transpiration in oil-sprayed plants obtained by substituting zero for Y in the calculated regression equation.

Variety	Regression Equation[a] $Y=a+bX$	s_b	$s_{y\,at\,Y=0}$	r	F for Slope df = 1,5	Expected Days for Recovery
Eureka lemon	=13.4−4.83326X	1.01	0.87	−0.90	22.77	25.2
Bearss lime	= 8.9−3.47299X	0.63	0.57	−0.93	30.39	15.5
Navel orange	=11.7−4.03667X	0.57	0.46	−0.95	49.75	32.5
Valencia orange	= 9.9−3.33055X	0.61	0.58	−0.93	30.19	38.4

[a] Y = mean difference in milligrams of water transpired per square centimeter of leaf surface per hour between paired untreated and sprayed plants; X = log10 of time in hours.

The standard errors of the mean differences (table 1) indicate considerable variability in transpiration of plants of the same lot. The variability appears to be of the same order in each of the varieties. The standard errors could be reduced by increasing the number of plants used; however, limitations in glasshouse space, and the amount of time required to weigh the plants, restrict the number of plants that can be used. The pretreatment mean differences in water transpired (table 1) indicate that in most instances the choice of plants for paired comparisons was good and that the members of the respective pairs of plants were comparable.

Although the standard errors are large, other evidence indicates that confidence may be placed in the mean differences (table 1) as estimates of the effect of oil spray on transpiration. The coefficients of correlation (r) shown in table 2 are all larger than required for significance at the 1% level. The F values for the slope of the lines obtained in analysis of variance of the variation about the response line also are larger than required for significance at the 1% level. The validity of recovery of transpiration in oil-sprayed plants with time is supported significantly by both the r and the F values obtained in calculations of correlations of log 10 of time vs. differences in water transpired between untreated and oil-sprayed plants.

The expected time for recovery of transpiration in the oil-sprayed plants may be calculated by substituting zero for Y in the regression equation. Values obtained thereby are listed in table 2 in days. These data indicate that Bearss lime recovered in less time after spraying than other varieties did. However, if s_Y is used and the respective ranges of expected days are calculated, differences between Eureka lemon and Bearss lime do not exceed the range of either variety, and differences between Eureka lemon and either navel or Valencia orange do not exceed the respective ranges of these varieties. Moreover, the value of b (the coefficient of X in the regression equations of table 2) for Bearss lime is less than that of b for either Eureka lemon or navel orange; this indicates that the rate of recovery is not faster in Bearss lime than in these varieties. If the standard errors of b (s_b), are considered, it may be seen that the differences in the various values of b are not greater than the standard errors. If regression lines are plotted from the respective equations, visual comparison suggests that the lines may be parallel and that such an adjustment would not exceed the limits of s_b. Within the conditions of the experiment it appears that the differences observed in recovery of transpiration of oil-sprayed plants of the several varieties are not more than might occur by chance. Comparisons of the transpiration data for Eureka lemon and Bearss lime do not indicate an explanation of the differences in leaf drop following applications of oil spray reported for these varieties under field conditions.

Cressman (1941) observed a rapid loss in the deposit of oil on lemon foliage sprayed in October with 1% light-medium oil—29% loss during the first afternoon and 42% by the morning of the second day after spraying. Ebeling (1950, p. 180) discusses the dissipation of spray oils from citrus foliage and cites evidence of losses of 80% of the oil deposited from 1.75% dosages applied in the fall—of light-medium oil in 17 to 25 days and of medium oil in 40 to 45 days. The rate of loss of oil may be associated with temperature and may have occurred at a faster rate in the glasshouse at 92° F. each day than in the field with variable temperatures from day to day.

Table 3.—Percentage reduction in transpiration in plants of several varieties of citrus during the first and third days after application of 1.75% medium-grade California emulsive spray oil in aqueous mixture.

Variety	Days After Spraying	
	1	3
Eureka lemon	66.3	38.4
Bearss lime	60.3	39.0
Navel orange	59.4	59.2
Valencia orange	68.4	55.0

Expected days for recovery of transpiration (see table 2) are in reasonable agreement with the time intervals cited above for the loss of spray oil from citrus foliage and suggest (1) that recovery of transpiration of oil-sprayed citrus foliage occurs with the loss of oil from the leaves and (2) that the reduction in transpiration following application of oil spray is due to physical interference by the spray oil on or in the leaf tissue.

The percentage reduction or inhibition of transpiration obtained by dividing the difference in weight of water transpired by untreated and oil-sprayed plants, by the weight of water transpired by the untreated plants, provides additional information on the relative effects of the oil-spray treatment on transpiration of the different varieties of citrus. Data on the percentage reduction in transpiration on the first and third days after application of the oil spray are given in table 3. Reduction in transpiration was greater on the first day than on the third day or any subsequent day, partly, no doubt, because the stomata were blocked with oil. In citrus leaves nearly all the stomata are on the underside of the leaf (Bartholomew 1931). The film of oil deposited by the spray spreads out over the surface of the leaf and then penetrates into the outer layers of cells. Movement of oil into leaf tissue continues only so long as there is free oil on the surface (Rohrbaugh 1941). Penetration is gradual, and some of the oil deposited may not penetrate the tissue during the first day after application. Openings of many stomata may thus be covered or blocked by free oil occurring irregularly in thin discontinuous films.

Generally, spreading and penetration of the oil deposited by an aqueous spray mixture containing a concentration of 1.75% oil are complete within 2 days after spraying. The percentage reduction in transpiration shown in table 3 for the first day after spraying indicates the dynamic effect, while the values for the third day represent the initial static effect of the oil deposit on transpiration. Although percentage reduction of transpiration in navel orange is as high on the third day as on the first day (see table 3), calculation of the correlation of transpiration vs. time indicates that recovery of transpiration in the navel orange plants occurred at a rate comparable to that in the other varieties.

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Sampling Methods Used to Determine the Prevalence of *Mansonia perturbans* During the Evening Peak Period[1]

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ABSTRACT

Observations on the prevalence of *Mansonia perturbans* (Walk.) were made during the evening crepuscular period in a pastured lowland at Beaverdam Swamp, Greenbrier, Alabama, in the summer of 1957. A 15-minute exposure period below 0.9 ft.-c. was used as standard.

Four methods of capture about man were utilized in an attempt to estimate attacking and non-attacking portions of the immediate mosquito population. In three of the methods captures of mosquitoes were made on the body and clothing of the collector by aspirator in conjunction with supplemental trapping devices including drop net, aerial net, and parked car to account for the resting and hovering remainder at the end of the period. In the fourth method intermittent aerial net sweeping was used.

A drop net device was developed consisting of three 7-foot legs connected by spacing rods at the base and supporting a circular ¼-inch iron pipe frame 48 inches in diameter with a trigger mechanism inside and with a folding cloth net 7 feet 4 inches long and 44 inches in diameter set outside and above. The net was hemmed to a 44-inch iron ring at the open end to provide an abrupt and even descent when the device was triggered.

In all, 141 male and 5,637 female *M. perturbans* were recorded in the nine tests from June 4 to July 10, inclusive. The males were distributed as follows: intermittent aerial sweeping, 109 (77.0%); aspirating in open with 10 terminal aerial sweeps, 22 (16.0%); and aspirating under net plus drop net catch and parked car technique each with 5 (3.5%). The 5,637 females collected in the four methods were represented as follows: aspirating under net plus drop net catch, 2,422 (43.0%); parked car technique, 1,242 (22.0%); intermittent aerial sweeping, 1,041 (18.5%); and aspirating in open plus 10 terminal aerial sweeps, 932 (16.5%).

The aspirator plus drop net and parked car technique recorded the highest numbers of females largely by providing confinable resting surfaces for mosquitoes deactivated in the proximity of the collector during the exposure period. The method employing 10 aerial sweeps at the end of the period gave the nearest estimate of the hovering remainder; however, the numbers varied greatly and appeared to reflect existing weather conditions more than population density. Intermittent net sweeping gave the best indication of males in flight and yielded useful background information on female flight activity when biting records were low. On moonlight nights approaching second quarter phase or full moon female mosquitoes freely entered the parked car. This was also true on cooler evenings in the alunar period.

Observations on the frequency of mosquitoes attacking man in the evening crepuscular (post sunset) period of activity near Beaverdam Swamp in 1956 indicated that capture by aspirator was adequate for determining relatively low numbers of *Mansonia perturbans* (Walk.) which rested and fed on man (Snow & Pickard 1957). However, it was recognized that no estimate was being made for those individuals that attacked en masse and were missed and those still hovering about the collector at the end of the earlier 15-minute collecting periods. Since these hovering mosquitoes were apparently disposed to attack during the time interval of the period, it was thought that this segment of the total attacking population should be included in future tests by means of supplemental capture techniques.

In previous work dealing with estimation of mosquito populations attacking man at night, capture of individuals in the immediate environs of the collector has been achieved mainly by netting devices. A netting device referred to as an umbrella trap has been developed for use by a single collector to capture insects normally congregating in a definite volume of air in his vicinity for a given length of time (Klock & Bidlingmayer 1953). A type of drop net called the "counting bell" was used in marshes of the Volga Delta to determine the types of biting Diptera attacking man after certain exposure periods (Montshadskij 1956). In the Tennessee Valley, the parked car technique has been regularly used at night as a trapping device for collecting mosquitoes attracted to man and/or light (Snow & Smith 1956).

METHODS.—Four methods of capturing mosquitoes in the 15-minute period were outlined for tests at Beaver-

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dam Swamp in 1957: (1) aspirating under drop net and drop net catch, (2) aspirating in open and 10 aerial sweeps, (3) parked car technique, and (4) intermittent aerial sweeping. The first three methods included biting and resting captures by aspirator on human hosts with supplemental captures at the end of a 15-minute exposure period. In the fourth method no supplemental catching was necessary since the catch was accumulated in the net until the end of the period.

On the basis of previous observations at Beaverdam Swamp, it was expected that the evening crepuscular peak (rise and fall) of mosquito activity would occur within 45 minutes after the 1-footcandle intensity had been reached. Since many of the mosquitoes became deactivated after the first 20 or 30 minutes and light conditions became poor for capture of mosquitoes by aspirator beyond 0.1 ft.-c., the 15-minute period following the 0.9 ft.-c. level was selected as standard. In all tests made the decline beyond 0.1 ft.-c. occurred during the 15-minute period and usually in the last 5 minutes. The collectors were known to be of equal efficiency in capturing mosquitoes by aspirator under light conditions in this range, but in extended collections below 0.1 ft.-c. individual differences might have influenced the results. It was also recognized that, once the drop net had fallen and the car doors had been closed in order to capture the residual populations, no further comparisons could be made with the other methods. By the time these residual collections in the drop net and parked car could be made (15 to 20 minutes) and resumption of collecting activity undertaken with all four methods participating, the peak of feeding activity would have passed.

EQUIPMENT.—The aspirator was of a standard type used by the Tennessee Valley Authority (TVA) for capture and retention of mosquitoes. The central or retaining portion consisted of a glass tube approximately 9½ inches long with an inside diameter of 1⅛ inches. The cork at the distal end was fitted with a glass tube about 4 inches long with an inside diameter of ⅜ inches. The tip of the glass tube was ground off at a slight angle and fire polished to facilitate capture of mosquitoes resting on vertical surfaces. The cork at the base of the central tube was also fitted with a 4-inch piece of ⅜-inch tubing and covered on the end projecting slightly inside the central tube by a piece of 100-mesh screen wire taped at the sides of the cork. A black rubber hose, 26″ long and ¼″ inside diameter, was fitted to the free end of the ⅜-inch tubing in the basal cork. Aspirators having a larger central tube overtax the lung capacity of the operator when attacking mosquito densities are high (20 per minute). Smaller central tubes fill up with mosquitoes too rapidly and are impractical from the standpoint of time lost in removing the mosquitoes and in reduced intake of air by plugging the screen wire filter.

A portable cloth drop net (fig. 1) was designed so that the collector could trigger the apparatus at the end of a selected exposure period, thereby confining himself and the mosquitoes still flying or resting under the canopy where the capture of this residual population could be completed. The frame of the device was constructed of

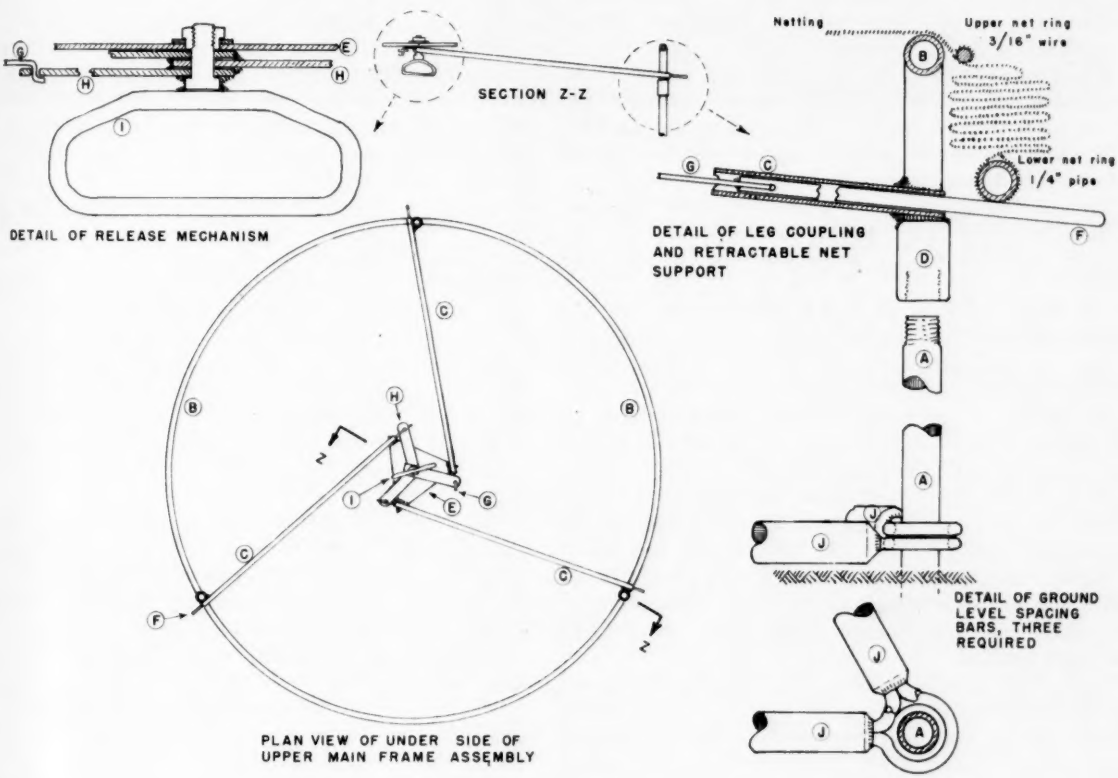
SECTION Z-Z
DETAIL OF RELEASE MECHANISM
Netting
Upper net ring
3/16" wire
Lower net ring
1/4" pipe
DETAIL OF LEG COUPLING AND RETRACTABLE NET SUPPORT
DETAIL OF GROUND LEVEL SPACING BARS, THREE REQUIRED
PLAN VIEW OF UNDER SIDE OF UPPER MAIN FRAME ASSEMBLY

FIG. 1.—Drop net device.

black iron pipe with nominal diameters of $\frac{1}{8}''$ and $\frac{1}{4}''$. It was supported by three $\frac{1}{4}$-inch iron pipe legs (A), 7 feet long and threaded at the upper end. The legs were coupled to an upper circular frame (B) of $\frac{1}{4}$-inch pipe 43 inches in diameter. The housing for the release mechanism situated within the upper frame consisted of three pieces of $\frac{1}{8}$-inch pipe (C) welded at their outward ends to the leg couplings (D) on the edge of the frame and at their inward ends to the apices of a 6-inch triangular iron plate (E) located in the middle of the frame. The release mechanism consisted of three 8-inch steel rods (F) ($\frac{1}{4}''$ diameter) attached individually by music wires (G) (80/1000″ diameter) to a three-point spider arrangement (H) with a knuckle type handle (I). The spider arrangement and handle were welded to a bolt passed through the center of the triangular plate (E) and secured by a shouldered nut. When the handle was turned as far as possible counterclockwise, the steel rods (F) were extended through the iron pipe housing about $1\frac{1}{2}$ inches beyond the upper frame so as to accommodate the folded drop net and ring in set position. Release was accomplished by a quick clockwise turn of the handle. The drop net was made of inexpensive veiling commonly used for skirt linings and was reinforced with $1\frac{1}{2}''$ cloth tape spaced about 24 inches apart. It was 7 feet 4 inches in length and 44 inches in diameter. At the open end the net was hemmed to a $\frac{1}{4}$-inch iron pipe ring also 44 inches in diameter. The ring served to guide the net evenly in release and added sufficient weight for an abrupt drop to the ground. Spacing bars (J) of $\frac{1}{4}$-inch pipe were also used on the legs at the ground for uniform spacing of the legs which provides clearance for the net. A ducking bag was provided for the net and ring to prevent tearing when in transport.

The aerial net used in the procedure for Method 4 was Ward's C2 type with a net bag 12″ in diameter, 28″ long, and of medium mesh Brussels netting. The net bag used in Method 2 at the termination of the collection period differed only in having medium mesh scrim netting.

The automobile used in the parked car collections was a black 1953 Chevrolet, 4-door sedan, series 150. The interior was upholstered with gray cotton headliner cloth.

Two light meters were used in conjunction with the measurement of light before and during the collection period. The General Electric PF-13 sensitive light meter was used mainly to determine the limits of the collection period (0.9 ft.-c. and below). It was scaled between 15 and 0.05 ft.-c. A Weston photometer, model 756, with a self-generating selenium photocell Luxtron No. 7-1A was calibrated with the PF-13 meter and was used mainly to measure uniformity of performance and light conditions at the collection site. The Weston unit was scaled well above the upper limit of the PF-13 scale (15 ft.-c.) and was used also to determine when collectors should be in position and when the upper range of the PF-13 meter had been reached.

Hygrothermograph readings were taken at the forest margin collecting site with a Friez recorder (Ser. 1667). Wind velocity was measured with a Taylor Biram's anemometer. Background information on the insect fauna in flight before and during the collection period was gathered by means of a rotary trap modeled after one used in Arkansas (Horsfall 1942).

PROCEDURE.—In the first method the collector was seated on a nail keg inside the tripod frame and therefore beneath the folded drop net such that about 30 inches of open space intervened between the collector's head and the overhead net. Attacking mosquitoes landing on the collector were captured by aspirator over the 15-minute period ranging below 0.9 ft.-c. At the end of the period the drop net was released by reaching up and turning the handle. This was done carefully so as not to disperse the hovering mosquitoes and those resting on the tripod frame. After the net had fallen in place, the mosquitoes already in the aspirator from the 15-minute collection were transferred to chloroform killing tubes, and the residual in the drop net was collected by aspirator with the aid of a flashlight.

The second method included biting and resting collections on the exterior of the body and clothing over the same 15-minute period but with 10 aerial sweeps about the head and body at the end of the period to collect hovering individuals.

The parked car technique was used as the third method. Car doors and windows were left open during the 15-minute period to allow mosquitoes free access to the interior of the car. The collector was seated in the back seat and captured the mosquitoes as they entered and attempted to bite or rest on the interior of the car. No exterior lights were used, but the domelight was left on and a flashlight was used to locate resting mosquitoes. At the end of the period the doors were closed and the mosquitoes remaining in the car were captured. Captures of biting and resting individuals in the parked car were not kept separate in the regular tests. It has been thought that if the resting mosquitoes were not caught during the collection period, some might leave the car before the end of the period and would not be represented in the total number that entered. To have adequately defined both attacking and resting groups of mosquitoes in the car, it would have been necessary to have allowed the resting mosquitoes to accumulate on the interior of the car until the end of the period. An additional test with this in mind was undertaken over a 45-minute period on the evening of August 12.

In the fourth method intermittent aerial net sweeping was employed by a standing collector. A sweep consisted of continuous net movement arcing above the head twice, once on each side of the body and across the front. Essentially a figure "8" was made overhead and across the middle. Approximately 30 sweeps were made in the 15-minute collecting period. Of necessity all mosquitoes were retained in the net until the end of the period and then chloroformed.

The collecting stations (fig. 2) were set at approximately 60-foot intervals in weed and short grass cover along the wooded south margin of the swamp. The collectors were in position usually by 7:00 p.m. or about 15 minutes before the expected onset of the evening crepuscular peak period. Occasional females of *M. perturbans* and *Aedes vexans* (Meig.) were captured before the 0.9 ft.-c. level was reached, but group activity of nocturnally active species was never recorded at the collecting stations above the 0.9 ft.-c. intensity in the immediate post sunset period. The rotary trap was placed in operation prior to the 15-minute collection period because a portable generator was used to supply current and additional

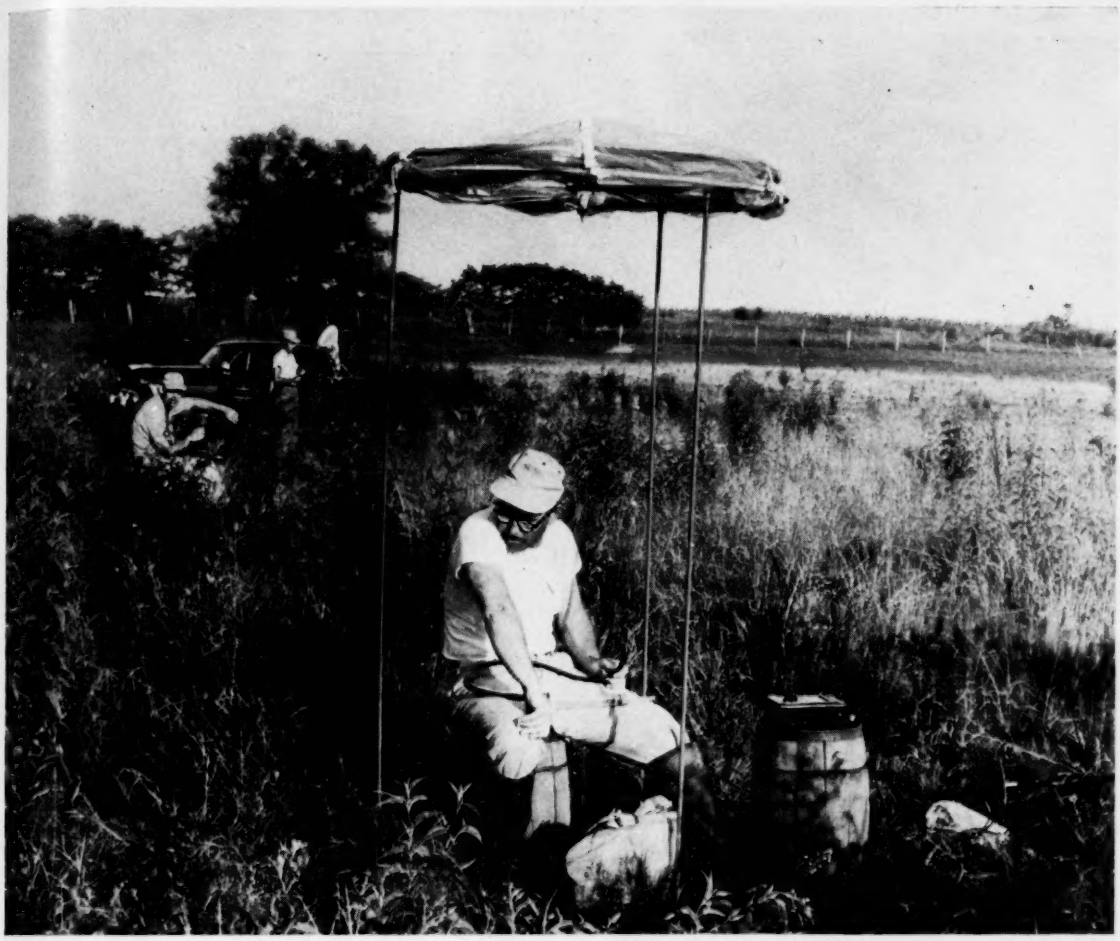

FIG. 2.—Sampling stations used for capture of *Mansonia perturbans* (Walk.) in crepuscular period along the south margin of Beaverdam Swamp, Greenbrier, Alabama, July 9, 1957.

manpower was not available to attach the chloroform jars on the screen wire cones and start the trap at the beginning of the period. The trap was stopped about 2 to 3 minutes after the end of the collection period.

The collectors wore khaki clothing and caps. In order to facilitate capture of mosquitoes and decrease annoyance to the collectors, the surface of the body was protected by clothing and cheesecloth and only the forearms, hands, and face were exposed.

RESULTS.—Numbers of adult *M. perturbans* captured by the four methods using a 15-minute collection period are given in table 1. In all, 141 male and 5,637 female *M. perturbans* were taken in nine tests from June 4 to July 10, inclusive. The male catch was distributed in descending order of abundance as follows: intermittent aerial sweeping, 109 (77.0%); aspirating in open with 10 aerial sweeps, 22 (16.0%); and aspirating under drop net plus drop net catch and the parked car technique each with 5 (3.5%). The 5,637 females taken in the four methods were distributed as follows: aspirating under drop net and drop net catch, 2,422 (43.0%); parked car technique, 1,242 (22.0%); intermittent aerial sweeping, 1,041 (18.5%); and aspirating in open and 10 aerial sweeps, 932 (16.5%).

The method of aspirating under drop net combined with drop net catch yielded the most uniformly high collections of total *Mansonia*. Total catches by this method ranged from 109 to 395. Comparative ranges were 64 to 196 for aspirating in the open with 10 aerial sweeps, 47 to 272 for the parked car technique, and 32 to 349 for intermittent aerial sweeping.

DISCUSSION.—Because weather conditions varied from test to test, a brief discussion of each test is presented below. Air movement, however, in all tests was insufficient to be measured by anemometer. The test on June 4 was accompanied by slight intermittent showers over a 19-minute exposure period below 0.9 ft.-c. of which 15 minutes was considered effective collecting time. Interruption of collecting was mainly due to wetting of equipment and disturbance of resting individuals by falling rain. Nevertheless, 1,102 *M. perturbans* taken in this test constituted 19% of the total for the nine tests. That the mosquitoes were restless is evidenced by the high count in the sweep nets over the small numbers feeding on the collector in Method 2. Even at the end of the period 7 males and 24 females were netted in 10 aerial sweeps, whereas only 70 had been taken on the body over the total period. A large number were localized under the drop net and the collector was able to aspirate 111 from the body. Mosquitoes readily entered the parked

Table 1.—Record of adult *Mansonia perturbans* taken about man by four sampling methods during a 15-minute crepuscular period below 0.9 footcandle, Beaverdam Swamp, Greenbrier, Ala., June 4–July 10, 1957.

	June												July							
	4[a]		6		11		12		18		25		2		9		10			
Weather:	Rainy		Clear		Clear		Clear		Cloudy		Clear		Clear		Clear		Clear			
Moon Visible:	None		8-Day Moon		13-Day Moon		None		None		None		5-Day Moon		12-Day Moon		13-Day Moon			
	75–72[b]		69–68		74–73		76–75		75[b]–74		65–64		69–68[b]		75–74[b]		63–61		Total	
Temp. Range, °F.:	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Method																				
Aspirating under drop net		111		113		126		155		81		94		51		70		82		883
Drop net catch		269	2	273	1	146	1	239		273	1	63		58		151		67	5	1339
Aspirating in open		70	1	183		67	1	118		110		97		64	1	81		62	3	852
Aerial sweeps (10)	7	24		12	10	7		19		2	1	6		2		7	1	1	19	80
Parked car technique	4	268		84		231		199	1	96		67		97		47		153	5	1212
Intermittent aerial sweeping	19	330	2	147	38	119	25	193	3	87	19	52	3	30		51		32	109	1041
Total per test	30	1072	5	812	49	696	27	923	4	649	21	379	3	302	1	407	1	397	141	5637

[a] Actual lapsed time was 19 minutes with effective collection time of 15 mintues.
[b] Temperature decline had not quite reached level indicated.

car during the rain and the count of 4 males and 268 females was the highest for all tests with this method. Collections from the rotary trap were notable in the comparatively high number of males in flight—the upper cones (7′) with 17 males and 4 females and the lower cones ($2\frac{3}{4}$′) with 12 males and 4 females.

Weather conditions on June 6 were clear, cool, and humid (94 to 95%) during the 15-minute period. An 8-day moon was near zenith. Mosquitoes were more active during the first half of the period in the 0.9–0.2 ft.-c. range. Some adults settled out on the legs of the drop net in the first 5 minutes of the period. This deactivation could account for high captures under the drop net and on the surface of the exposed collector in Method 2. However, mosquitoes were definitely reluctant to enter the parked car in the observation. Intermittent net sweeping showed much less than half of the density mosquitoes on the wing as on June 4.

Observations on June 11 were undertaken with a 13-day moon between 10° and 15° above the eastern horizon. Conditions were fairly close and calm. The feeding activity was moderate over the 15-minute period, but the mosquitoes appeared wary in the moonlight and were difficult to catch by the collectors in the open. Entry of the parked car was high. Male mosquitoes were quite active during the period and terminal aerial net sweeps showed 10 to be congregated over the head of the collector at the end of the period. Intermittent aerial sweeping also showed a relatively high number of males in flight in the 15-minute interval below 0.9 ft.-c.

On June 12 the weather was calm and clear with no direct moonlight (full moon) until after the collection period. Relative humidity remained in the mid-80% range. Notable activity of *M. perturbans* commenced at about 7:23 p. m. (0.9 ft.-c.) and was intense after 7:33 p.m. (0.2 ft.-c.). Drop net counts were high and net sweeping showed ample numbers were in flight. Darker conditions than on the previous evening were thought to be the main factor in higher captures with aspirator by exposed collectors.

In the test on June 18 conditions were close with relative humidity from 90 to 92% and ground mist was scattered through the vegetation. Attack of mosquitoes was not pronounced until the 0.4 ft.-c. level was reached 3 minutes after the onset of the period (0.9 ft.-c.). Localization of mosquitoes under the drop net was high. Females were unsettled about the collector and were generally down in numbers according to aerial sweeping results.

Temperature conditions in the 65 to 64° F. range over the observation period from 7:27 to 7:42 p.m. on the evening of June 25 resulted in a marked curtailment of flight and feeding activity in the collecting stations. Drop net recoveries (1 male, 63 females) were the lowest in all tests with this method; however, the aspirator and drop net still registered the highest catch on June 25. Captures by intermittent aerial sweeping showed a relatively high percentage (26.8) of males in flight. Rotary trap records over the interval from 7:10 to 7:45 p.m. suggested that some *M. perturbans* may have passed over the collection site (swamp margin) in an upper warmer air stratum to situations in open fields usually known to be several degrees warmer for a short period just after dark (Snow & Pickard 1957). The lower cones ($2\frac{3}{4}$′) yielded 2 males and 18 females, whereas the upper cones (7′) retained 1 male and 33 females.

The test on July 2 was accomplished under cool, clear, and moist conditions with a 5-day moon high in the western sky. A ground mist formed along the margin about 15 minutes before the period commenced. Relative humidity rose from 95 to 96% during the period. Flight and feeding activity in the open was slight with very low numbers in the drop net and sweeping collections. Results were better in the parked car where higher temperatures may have been an inviting factor.

Higher temperatures on the evening of July 9 appeared to encourage greater activity during the crepuscular period in the open, although density of *M. perturbans* had generally fallen off since midJune. Fairly warm and humid (92% relative humidity) conditions seemed to discourage mosquitoes from entering the parked car readily in the early evening collection period, 7:26 to 7:41 p.m. For about 20 minutes after the end of the collection period, adults of *M. perturbans* remained inactive on vegetation. Warm dry air moving in from nearby cottonfields resulted in an upsurge of flight and feeding

activity shortly after 8:00 p.m. The hygrothermograph record showed that in 10 minutes the temperature had risen from about 74° to 76° F. and the relative humidity had dropped from nearly 94% to 87% with the sharpest change in the first 4 minutes. A 12-day moon was present in the eastern sky over the observation period.

The final test using the four methods together was carried out on July 10 with a 13-day moon in the eastern sky between 10° and 20° above the horizon. Temperatures were considerably lower than on the previous evening and heavy condensation of vapor on surrounding vegetation occurred during the period. Mosquitoes completed their main peak of feeding in the 15-minute period below the 0.9 ft.-c. point. Numbers in the parked car were over three times higher than on the previous evening. Sweeping showed a small number in flight. The residual in the drop net was down over half of the July 9 collection.

While the drop net and parked car technique rated high in overall catching ability, a separation of the mosquitoes taken in resting position by the two methods was not possible in the selected 15-minute period since some were still in flight when the net was dropped and the car closed for collection of the residual. An additional observation was made on August 12 employing only these two techniques. The collection period was extended to 45 minutes so as to minimize the possibility of mosquitoes being in flight about the collectors. Mosquitoes were collected only on the body surface until the end of the period when the resting captures were made. Moonlight which might favor the parked car method was absent. No domelight was used inside the car. Temperatures ranged from 74° to 72° F. over the period. Light intensity dropped from 0.9–0.1 ft.-c. in the first 9 minutes of the test. Results are given in table 2. The low numbers recovered in the test were attributed to cotton dusting operations which had been undertaken in the areas since the first week in August. A significant difference was shown in the number of female *M. perturbans* found resting on the drop net surfaces (114) and on the interior of the car (57). It is mainly a reflection of the tendency for *M. perturbans* to frequent open situations and rest on exposed surfaces. The specific preference among mosquitoes for exposed and protected resting sites is more apparent when the comparison is extended to *Anopheles quadrimaculatus* Say which usually feeds and rests in sheltered situations. It will be noted that of the 55 specimens taken in the test, 49 were collected in the car. Another important observation revealed in the test on August 12 related to small number of mosquitoes in the car actually feeding on the human host.

CONCLUSIONS.—In exposed situations the drop net and frame was found to be a particularly useful mechanism for localizing *M. perturbans* deactivated in the crepuscular activity period and for trapping mosquitoes still hovering about the collector. The presence of the collector beneath the net was necessary to congregate numbers on the interior surface. Adults of *M. perturbans* were also observed to rest on the outside and top of the net as well as on nearby vegetation but never so concentrated as on the underside of the net. Since some mosquitoes were always hovering around the collector after the 15-minute exposure period, it was not possible to separate the hovering numbers from resting individuals in the total net catch. When the exposure time was extended to 45 minutes, the peak of early evening feeding had subsided and a clear separation was possible between those resting on the drop net surfaces and those taken on man.

The 10 sweeps used at the close of the 15-minute period in Method 2 was the only procedure actually separating the residual mosquitoes from those captured by aspirator during the period. Weather conditions and population density were important factors in determining the numbers about man at a given moment, but the terminal sweeps were of definite value in estimating attractivity of mosquitoes to man when exposure periods were so timed that a hovering group persisted. In the tests at Greenbrier in 1957 the hovering groups were quite low as compared with previous years. However, the test with this method on June 4 under rainy conditions does show that a fairly good percentage of the mosquitoes about man would have been excluded from the total without net sweeping.

The use of the parked car technique as outlined for Method 3 was quite effective for the capture of *M. perturbans* and, like the drop net, provided an immediate resting surface to detain deactivated individuals for immediate or subsequent capture. The presence of the collector in the car appeared to account for mosquitoes staying in the car as feeding or resting individuals. When collectors have remained outside the vehicle during the peak activity period, adults of *M. perturbans* have not been found remaining inside. In one test when the exposure period was extended to 45 minutes and most of the attacking mosquitoes had become deactivated, a high resting population of *A. quadrimaculatus*, a species known to seek sheltered situations, was found in the car. While the parked car technique retained good numbers of *M. perturbans*, it appeared to be a more sensitive indicator of those preferring to feed and rest in enclosed situations.

Collections of *M. perturbans* in the parked car were especially high on cool evenings and on nights when moonlight was pronounced. High counts in the car were also noted on one rainy evening. When warm and humid conditions were present in the early part of the evening

Table 2.—Record of female mosquitoes taken by two sampling methods using human hosts in a selected 45-minute crepuscular period, Beaverdam Swamp, Greenbrier, Alabama, August 12, 1957.

METHOD	SPECIES	NO. BITING MAN			RESTING RESIDUAL	TOTAL
		7:01–7:16	7:16–7:31	7:31–7:46		
Human host and drop net	*Anopheles quadrimaculatus*	♀ 2	1	1	♀ 2	6
	Mansonia perturbans	7	♀ 2	♀ 2	114	125
Human host in parked car	*Anopheles quadrimaculatus*	9	4	0	36	49
	Mansonia perturbans	12	7	0	57	76

activity period, *M. perturbans* were quite hesitant to enter the car.

Intermittent aerial sweeping used in Method 4 yielded the best information on the presence of male *M. perturbans* and gave a fair indication of female abundance when considered with those attracted to the collector in Method 2 where limited net sweeping was used. The high number (349) netted under rainy conditions on June 4 when mosquitoes appeared too agitated to feed readily suggests that this method may be of particular value in providing background information on mosquito density and in giving some measure on the effects of certain atmospheric conditions. As such, it indicated considerably decreased crepuscular activity of adult mosquitoes in the lower temperature ranges. One disadvantage of this method concerned excessive descaling of the mosquitoes retained in the net over the 15-minute period. While *M. perturbans* was readily recognizable, it should be noted that where intact notal patterns are desirable for identification continual net sweeping may have limited use.

In selecting methods for appraisal of adult mosquitoes attacking man, it is necessary to relate the procedures to certain points of mosquito behavior. The diel cycle of activity should be determined for species to be studied in a particular locality. Under certain conditions mosquitoes may be more abundant at dawn than at dusk (post sunset) but not adequately sampled by biting collections or landing rates because the females are more disposed to seek shelter than to feed at this time (Snow & Pickard 1957). Another important point in mosquito behavior is the tendency of a species to feed more readily in the open or to enter dwellings and situations with a darkened interior for this purpose.

Considering that the drop net device can be transported in a sedan vehicle and readily assembled and disassembled in the field, it is regarded as the method of choice in conjunction with the parked car technique. Both procedures are capable of isolating a particular environmental situation at a prescribed time and in addition to estimating density may provide some measure of the mosquito's tendency to feed and rest under exposed or enclosed conditions.

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Application of the Colorimetric Whole-Blood Method to the Measurement of Bovine Red-Blood-Cell Cholinesterase Activity[1]

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ABSTRACT

A rapid and precise colorimetric method for the determination of bovine red-blood-cell (RBC) cholinesterase (Ch E) activity, employing whole blood as the enzyme source, is presented. Whole-blood determinations on 110 animals of mixed sexes of varied ages and breeds demonstrated a mean activity of 1.47 ±0.22 micromoles of acetylcholine (ACh) hydrolyzed by 50 microliters of whole blood, incubated for 10 minutes at 37° C. with a substrate concentration of 0.002 *M* acetyl choline chloride (total substrate 4 micromoles). Under these conditions nearly 96% of the Ch E activity of whole blood is due to RBC Ch E. The effect of both the time and the blood volume (enzyme concentration) on hydrolysis of ACh was determined and a linear relationship was found to exist beyond the 50 microliters volume of whole blood and 10-minute time interval employed in this study. The method gives good agreement with the electrometric RBC method in measuring *in vivo* inhibition by organophosphorus insecticides.

The increasing use of the organophosphorus insecticides for the control of insects affecting domestic animals and forage crops has occasioned the need for a rapid and precise method for the determination of bovine Ch E[2] activity. Inasmuch as the Ch E activity of red blood cells (RBC) may be employed as an index of nervous system Ch E activity, such a method would serve as a means of diagnosing poisoning by these anti-Ch E agents as well as a tool in residue-ingestion and mode-of-action studies.

Several adequate methods for the determination of Ch E activity, including manometric (Ammon 1933),

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[2] The following abbreviations are used through the text: Ch E for cholinesterase, ACh for acetylcholine, and RBC for red blood cells.

electrometric (Michel 1949), and titrimetric (Brown & Bush 1940) techniques, are available.

Metcalf (1951) applied the colorimetric reaction of Hestrin (1949) for acetylcholine to the measurement of human RBC and serum Ch E activity. The advantages of this method have been discussed by Metcalf (1951) and Fleisher & Pope (1954). Briefly they are (1) preciseness and reproducibility, (2) direct measurement of unhydrolyzed substrate, (3) rapidity, (4) small volume of blood required, and (5) suitability for determining a low degree of Ch E activity following inhibition.

More recently Fleisher and coworkers (1954, 1955) have employed the colorimetric method for determining the Ch E activity of hemolyzed whole blood, taking advantage of a low substrate concentration to favor the activity of the RBC "specific" Ch E over the serum Ch E, so that the activity of the whole blood reflects preferentially the activity of the RBC Ch E. This modification eliminates the time-consuming procedure of separating serum and RBCs. Inasmuch as bovine plasma is practically inactive toward choline esters (Augustinsson 1948, Radeleff & Woodward 1956), the whole-blood method was believed to be especially applicable to the measurement of the Ch E activity of bovine RBCs.

The method of Fleisher and coworkers (1954, 1955) has been adapted for bovine whole blood and the technique compared with the electrometric method for measuring *in vivo* inhibition.

REAGENTS.—(1) Phosphate buffer, 0.134 *M*, pH 7.2: prepared by mixing 7 parts (v/v) of a solution of 23.752 grams of $Na_2PHO_4 \cdot 2\ H_2O$ per liter and 3 parts of a solution of 18.156 grams of KH_2PO_4 per liter; pH adjusted to 7.2 if necessary.

(2) Acetylcholine, 0.04 *M:* prepared with 0.7266 gram of acetylcholine chloride in 100 ml. of 0.001 *M* acetate buffer, pH 4.5.

(3) Acetylcholine, 0.004 *M:* reagent 2 diluted with 9 volumes of phosphate buffer (reagent 1); made daily in quantity required for analyses.

(4) Hydroxylamine hydrochloride, 2 *M;* 27.8 grams dissolved in distilled water to make 200 ml., discard after 2 weeks.

(5) Sodium hydroxide, 3.5 *M;* 28 grams dissolved in distilled water to make 200 ml.

(6) Alkaline hydroxylamine; equal volumes of reagents 4 and 5, mixed shortly before use in quantity required for samples being analyzed.

(7) Hydrochloric acid; concentrated acid sp. gr. 1.18, diluted with 2 parts by volume of distilled water.

(8) Ferric chloride, 0.37 *M;* 10 grams of $FeCl_3 \cdot 6\ H_2O$ to 100 ml. in 0.1 *N* hydrochloric acid.

Reagents 1 through 4 should be kept refrigerated when not in use.

PREPARATION OF STANDARD CURVE.—Aliquots of 0.25, 0.50, 0.75, and 1.0 ml. of 0.004 *M* ACh solution (reagent 3) were pipetted into 18×150 mm. standard Pyrex test tubes, and then made up to 2 ml. by adding phosphate buffer (reagent 1). Four milliliters of alkaline hydroxylamine (reagent 6) were added to each tube and swirled vigorously. After 2 minutes 2 ml. of the acid solution (reagent 7) was added to the sample, followed by 2 ml. of the ferric chloride solution (reagent 8), with thorough mixing after each addition. The solutions were filtered through Whatman No. 40 filter paper into cuvettes, and the density of the brown color formed was determined at 540 millimicrons with a spectrophotometer 10 minutes after the addition of the ferric chloride. A blank correction for nonspecific color was obtained in the same manner by using 1 ml. each of 0.004 *M* ACh solution and phosphate buffer, except that the order of addition of the alkaline hydroxylamine (reagent 6) and hydrochloric acid solution (reagent 7) was reversed. The per cent transmission values were converted to optical density and plotted against the micromoles of ACh present in each sample, resulting in a curve similar to that shown in figure 1. The curve was used as a standard for the conversion of optical-density values to micromoles of ACh.

DETERMINATION OF WHOLE-BLOOD CH E ACTIVITY.—Fifty microliters of heparinized bovine whole blood was pipetted into a test tube containing 0.95 ml. of 0.01% saponin solution, and the micropipet was rinsed three times into the saponin solution. The hemolyzed whole-blood sample was placed in a clinical water bath, adjusted to 37° C., and allowed to equilibrate for 5 minutes. Then 1 ml. of 0.004 *M* ACh solution was added to the tube, thoroughly mixed by swirling, and the time of substrate addition noted. A control sample of 1 ml. of 0.004 *M* ACh was incubated in a separate tube for nonenzymic hydrolysis determination.

After exactly 10 minutes the reaction was stopped by the addition of 4 ml. of alkaline hydroxylamine. Follow-

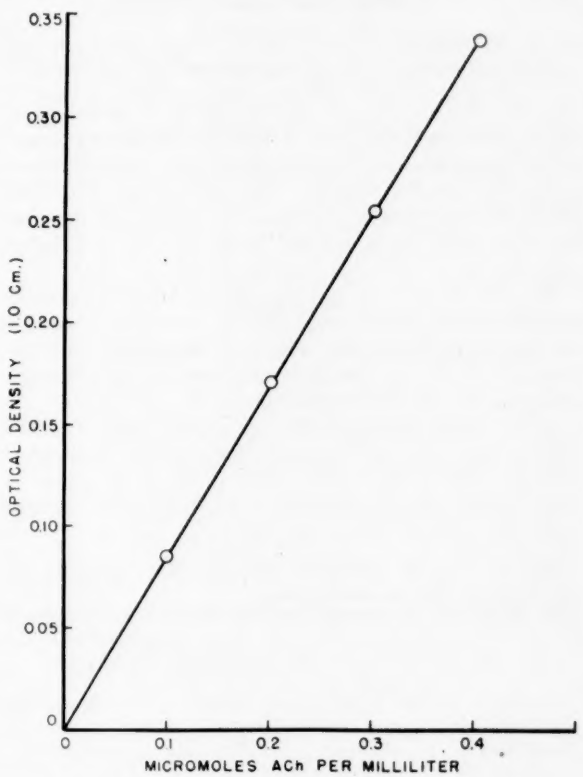

FIG. 1.—Standard curve for ACh. Beckman DU spectrophotometer. 540 millimicrons. 1.0-cm. light path.

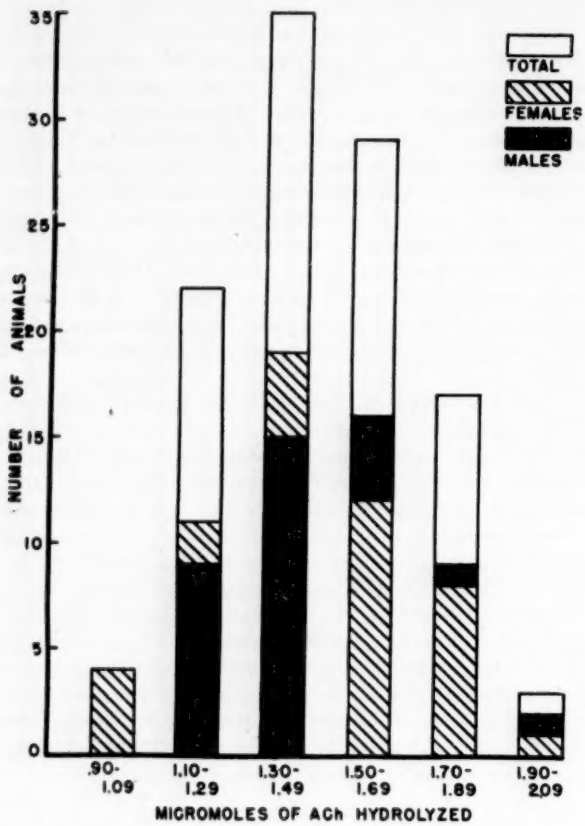

FIG. 2.—Distribution of normal whole-blood Ch E-activity values in mixed sexes, cows, and bulls of different ages and breeds.

ing its addition to the control sample, 1 ml. of hemolyzed blood solution, containing 50 microliters of whole blood in 0.01% saponin solution, was added, and the color developed and read as previously described. All readings were corrected for nonspecific color by use of a blank correction.

The Ch E activity (micromoles of ACh hydrolyzed) was calculated by subtracting the micromoles of ACh remaining in the sample following incubation with the enzyme from the micromoles of ACh in the control sample following nonenzymic hydrolysis.

POPULATION SAMPLE.—The mean and range of values for the RBC Ch E activity of normal cattle as determined by the colorimetric whole-blood method are given in table 1. The mean, determined for 110 animals of mixed

Table 1.—Ch E activity of cattle as determined by the colorimetric whole-blood method.

SEX	NUMBER OF ANIMALS	MICROMOLES OF ACh HYDROLYZED	
		Mean	Range
Mixed	110[a]	1.47±0.22	0.96–2.02
Bulls	51	1.52±0.20	1.14–1.98
Cows	55	1.44±0.23	0.96–2.02

[a] 51 bulls, 55 cows, 4 steers.

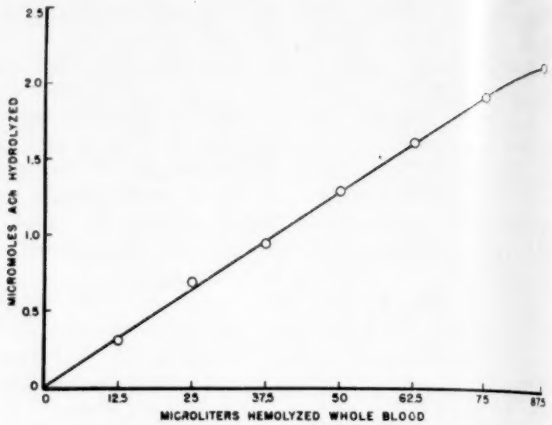

FIG. 3.—Relation of Ch E activity to quantity of whole-blood employed.

sexes and varied ages and breeds, was found to be 1.47 ±0.22 micromoles of ACh hydrolyzed. It was slightly higher for bulls than for cows. The frequency distribution of the Ch E activity of mixed sexes, cows, and bulls is shown in figure 2.

PRECISION.—To check the reproducibility of the method 10 replicate determinations were made on a single blood sample. As shown in table 2, the precision was found to be 2.6%.

ENZYME CONCENTRATION AND TIME VERSUS HYDROLYTIC ACTIVITY.—The effect of the quantity of whole blood used on the hydrolysis of ACh is shown in figure 3. The results indicate that a linear relationship exists well beyond the 50 microliters employed in these studies.

The effect of reaction time on ACh hydrolysis was also investigated, and the results indicate linearity beyond the 10-minute time interval employed (fig. 4).

RELATIVE CONTRIBUTION OF SERUM AND RBC TO WHOLE-BLOOD CH E ACTIVITY.—To determine the relative contribution of RBC and serum ChE, following hematocrit determination, samples of blood were centrifuged in graduated tubes and the serum was removed. The RBC's were washed twice with a 0.9% sodium chloride solution and recentrifuged each time. The RBC's were made up to the original blood volume with

Table 2.—Reproducibility of colorimetric whole-blood method for measuring bovine Ch E activity.

SAMPLE NO.	MICROMOLES OF ACh HYDROLYZED
1	1.29
2	1.29
3	1.34
4	1.30
5	1.31
6	1.39
7	1.30
8	1.34
9	1.37
10	1.30
Mean	1.32±0.035
Precision	2.6%

Table 3.—Relative contribution of RBC and serum Ch E to bovine whole-blood Ch E activity.

Hematocrit	Whole-blood	RBC		Serum	
	Micromoles of ACh Hydrolyzed	Micromoles of ACh Hydrolyzed	Per Cent of Whole-blood Activity	Micromoles of ACh Hydrolyzed	Per Cent of Whole-blood Activity
Cows					
36	1.56	1.49	95.5	0.02	1.3
42	1.58	1.57	99.4	.03	1.9
36	1.22	1.15	94.3	.03	2.5
35	1.55	1.52	98.1	.10	6.4
35	1.33	1.29	97.0	.02	1.5
Bulls					
38	1.82	1.69	92.9	.16	8.8
42	1.52	1.47	96.7	.06	3.8
31	1.57	1.46	93.0	.14	8.9
38	1.98	1.88	94.9	.08	4.0
40	1.40	1.32	94.3	.10	7.1
Mean	—	—	95.6	—	4.6

Table 4.—Comparison of electrometric RBC and colorimetric whole-blood methods for measuring *in vivo* inhibition of bovine Ch E.

Sample No.	Per Cent Control Activity	
	Electrometric RBC	Colorimetric Whole-Blood
1	67.3	71.9
2	63.2	67.2
3	67.3	68.8
4	94.2	95.2
5	99.0	101.6
6	93.3	98.4
7	80.9	86.4
8	87.2	85.5

0.9% sodium chloride solution, and equivalent volumes of whole blood, suspended RBC's, and serum were assayed for Ch E activity. The average serum contribution for the 10 animals studied was less than 5%, with a range of 1.3 to 8.9%, of the whole-blood Ch E activity (table 3). A mean of 95.6% of the total Ch E activity determined by the method described in this paper was due to RBC Ch E activity.

COMPARISON OF COLORIMETRIC WHOLE-BLOOD AND ELECTROMETRIC RBC TECHNIQUES FOR MEASUREMENT OF *in Vivo* INHIBITION.—Measurements of blood Ch E activity of several animals treated with organophosphates were made by both the colorimetric whole-blood method and the electrometric RBC method of Michel (1949). The results in table 4 indicate good agreement between the two methods, with slightly higher values obtained by the colorimetric technique.

DISCUSSION.—The colorimetric whole-blood method appears to be especially suitable for the measurement of bovine RBC Ch E activity. In addition to eliminating the time-consuming operation of centrifuging, washing, and separating the RBC's, it is more rapid than the electrometric technique usually employed for bovine RBC determinations (Dahm & Jacobson 1956, Radeleff & Woodward 1956), requiring an overall time of 25 minutes for an analysis. By use of a planned schedule the authors are able to perform about 12 analyses an hour. Such a technique would be especially useful in large-scale programs involving treatment of cattle with organophosphorus insecticides.

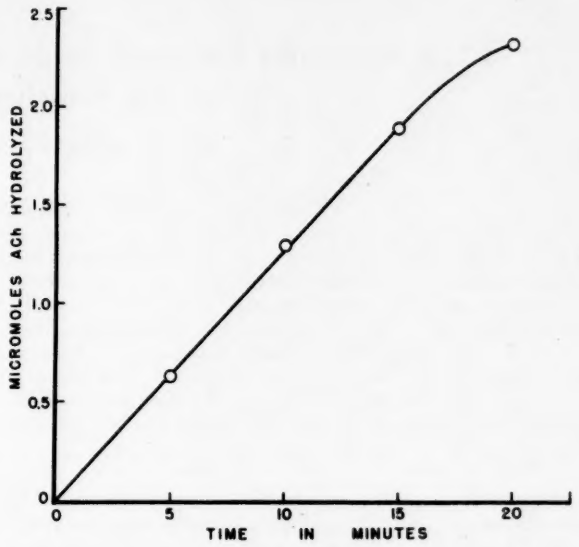

FIG. 4.—Whole-blood Ch E-activity as a function of time of reaction with substrate, employing 50 microliters of hemolyzed whole blood.

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Plum Curculio Populations in an Unsprayed Peach Orchard in Southern Illinois[1]

STEWART C. CHANDLER[2]

ABSTRACT

Weekly jarrings for five seasons showed a great reduction of adults of *Conotrachelus nenuphar* (Hbst.), from the overwintering to the first generation during 2 drouth years and 1 year of total crop loss from freeze. Total numbers jarred for the season dropped from 495 on five trees in 1953 to 36 in 1956, but with a return to more nearly normal moisture conditions and an excess of moisture in the last year of the study, 338 were jarred in 1957. Infestations at harvest followed a similar pattern, with 88% of the fruit injured in 1953, 8% in 1956, and 50% in 1957.

As a part of a study of a neglected peach orchard, the populations of the plum curculio, *Conotrachelus nenuphar* (Hbst.), as determined by jarring, were recorded over a 5-year period to study the occurrence throughout the season when undisturbed by spraying or other orchard operations, to correlate populations with flower and fruit development, and to learn how these populations are affected by weather conditions.

The orchard is located at Mounds in the extreme southern end of Illinois, at approximately latitude 37, and well within the area where there are two broods of plum curculios per year. It had received no sprays or dusts since the end of the crop year of 1951. Considering the neglect, fair crops have been produced, due to original fertility of the soil and to pasturing with cattle. Due to inefficient spraying or dusting and to rugged terrain which afforded good hibernating quarters, populations of plum curculios frequently had been high before the orchard was abandoned.

METHODS.—Five trees were jarred at approximately weekly intervals from bloom to almost harvest, using the same trees each year, if the crop was sufficiently large. Just before harvest insect and disease records were made. The data taken are summarized in tables 1 and 2, and the population curves are shown in figures 1 to 5, inclusive.

It is well known that hot, dry weather, especially during pupation and emergence of the adults in midseason, reduces the emergence of first-brood adults. Since it would appear from the records that June and July are the critical months for this transformation in the area, precipitation records taken at the Weather Bureau Station at Cairo, about 8 miles south of the orchard, are given in table 3.

RESULTS.—This study appears to show the following facts on the life history of the plum curculio:

1. Under normal conditions, undisturbed by man, the insect in this area emerges from winter quarters and appears on peach in late March or early April some time during the bloom. In years of light infestation jarring indicates that first appearance is delayed until the petal fall or shuck-crack periods. However, this may be more apparent than real, and may be due to scarcity of the insect.

2. The peak of occurrence of this overwintering generation is some time in May, from 4 to 6 weeks after full bloom, after which there is usually a sharp drop in numbers.

3. The first generation adults begin to appear in late June or early July from 7 to 9 weeks after the peak of the overwintering generation.

[1] Accepted for publication December 5, 1957.

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Table 1.—Numbers of plum curculios jarred from five unsprayed peach trees at Mounds, Ill., from 1953 to 1957, inclusive.

1953			1954			1955			1956			1957		
Date	No.	Fruit Development[a]	Date	No.	Fruit Development	Date	No.	Fruit Development	Date	No.	Fruit Development	Date	No.	Fruit Development
3/24	5	60% P.F.	3/15	0	50% Buds pink	3/14	0	83% B.O.	3/27	0	All pink	4/2	0	53% B.O.
3/31	8	90% P.F.	3/20	0	53% B.O.	3/16	0	95% B.O.	4/2	0	90% B.O.	4/9	1	62% P.F.
4/13	19	80% S.C.	3/24	0	70% B.O.	3/22	0	Freezes	4/5	0	90% P.F.	4/18	4	100% P.F.
4/22	2	40% S.O.	3/29	2	35% P.F.	3/29	0	3/14–16	4/12	0	100% P.F.	4/23	21	35% S.C.
4/28	40	100% S.O.	4/3	0	80% P.F.	4/12	4		4/18	1	S.C.	4/30	14	All S.O.
5/5	65	Diam. ¼ in.	4/6	2	100% P.F.	4/18	0		4/25	3	75% S.C.	5/7	19	Diam. ⅛ in.
5/12	95	Diam. ¾ in.	4/13	20	S.C.	4/26	7		5/3	0	75% S.O.	5/14	35	June drop started
5/19	104		4/20	22	S. most off	5/2	19		5/9	0	Diam. ¾ in.	5/21	14	
5/26	19		4/27	21	100% S.O.	5/16	23		5/16	3		5/28	22	
6/1	13		5/4	11	Diam. ¾ in.	5/23	6		5/21	3		6/4	17	
6/8	1		5/10	32		5/31	6		5/28	4		6/11	13	
6/17	10		5/17	14		6/6	6		6/5	0		6/18	22	
6/22	4		5/24	10		6/13	3		6/11	0		6/25	33	
6/30	11		5/31	10		6/21	0		6/18	2		7/2	34	
7/7	31		6/7	3		6/28	1		6/28	1		7/9	23	
7/13	13		6/14	2		7/5	0		7/2	4		7/15	28	
7/21	48		6/22	3		7/11	1		7/10	2		7/22	19	
7/28	4		6/28	5		7/22	0		7/17	4		7/30	16	
8/4	3		7/6	2		7/30	0		7/25	8				
			7/12	1					7/13	1				
			7/23	7										
			7/28	1										
Total	495			168			77			36			335	

[a] P.F., Petal fall; B.O., Blossoms open; S.C., Shucks cracked; S.O., Shucks off.

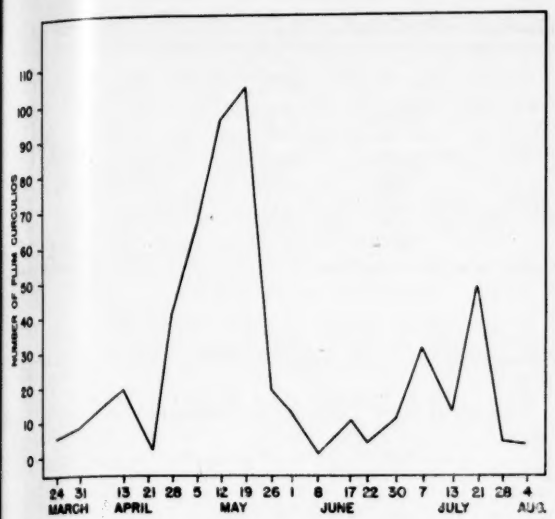

FIG. 1.—Numbers of plum curculios jarred from five unsprayed peach trees, Mounds, Ill., 1953.

4. The scarcity or abundance of first generation adults in any season may greatly affect the population the following year, and moisture conditions during the pupation and emergence period are an important factor in the production of the first generation.

5. Lack of a fruit crop, with or without favorable weather conditions, will greatly reduce the numbers of curculios the following year.

The great reduction in total numbers of curculios jarred from 1953 to 1954 is shown in table 1. This is correlated with the sizes of the first generation peaks in relation to those of the overwintering generation. It is interesting to note from the graphs that in 1955 the pattern of occurrence of the overwintering adults on the trees appears to be much the same as in other years despite the fact that no live fruits could be found in the orchard after the blooming period. These beetles may have fed on the buds and foliage but finding no fruit in which to oviposit, produced few if any progeny. Under these circumstances the increased precipitation (see

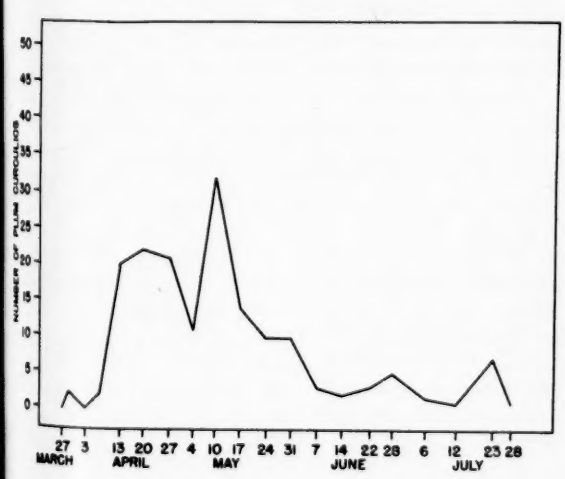

FIG. 2.— Numbers of plum curculios jarred from five unsprayed peach trees, Mounds, Ill., 1954.

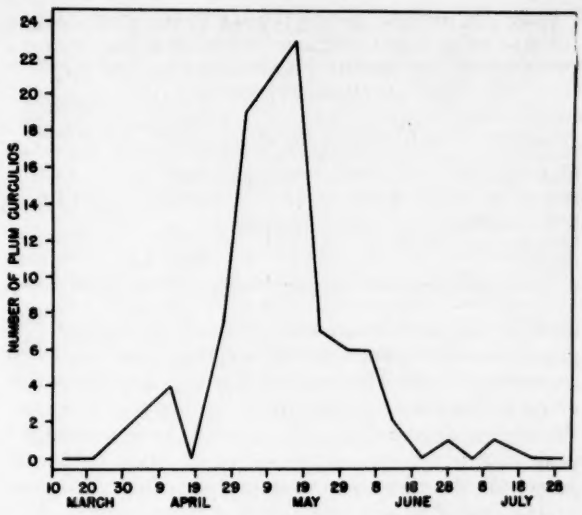

FIG. 3.— Numbers of plum curculios jarred from five unsprayed peach trees, Mounds, Ill., 1955.

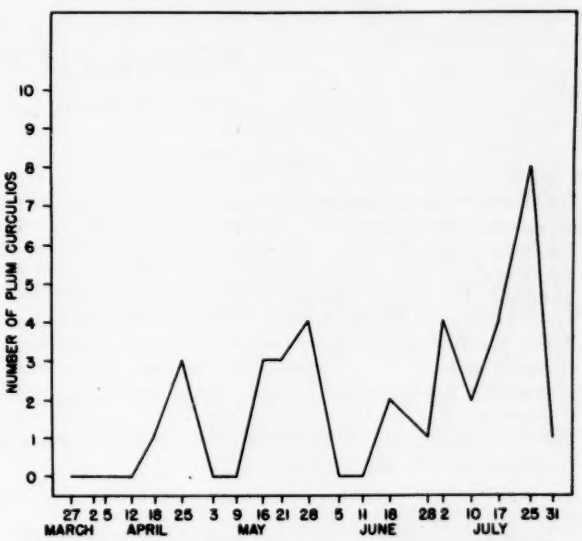

FIG. 4.—Numbers of plum curculios jarred from five unsprayed peach trees, Mounds, Ill., 1956.

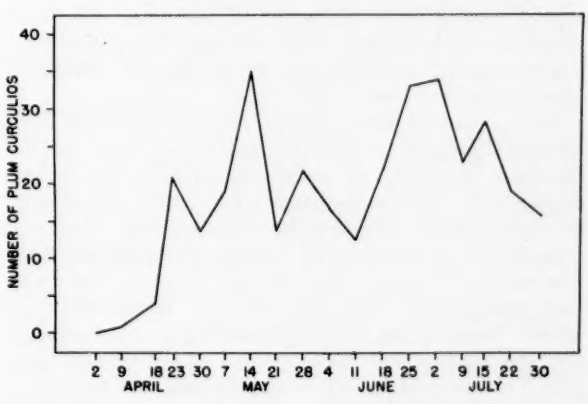

FIG. 5.—Numbers of plum curculios jarred from five unsprayed peach trees, Mounds, Ill., 1957.

Table 2.—Per cent of fruit injured by the plum curculio and infected by brown rot at harvest. Mounds Ill.

Year	Plum Curculio			Brown Rot
	Wormy	Stung	Total	
1953	—	—	88.8	55.2
1954	9.8	12.0	21.8	2.0
1955 (no crop)				
1956	7.2	1.4	8.6	0.4
1957	—	—	50.0	75.0

Table 3.—Precipitation at Cairo, Illinois, June and July.

Year	Rainfall (Inches)	
	June	July
1953	2.21	1.83
1954	3.87	3.26
1955	4.72	8.19
1956	5.61	2.36
1957	9.13	5.29

table 3) had little effect, and of the total number (77) jarred for the season, only two could be considered first generation adults. This resulted in a very low population in 1956, the lowest during the 5-year period. However, the increased rainfall in 1956 seems to have produced a peak in July twice as high as that in May. Of the 36 jarred for the season, 20 were probably first generation beetles, and it is very probable that these, carrying over into 1957, aided by the excessive rainfall that year, were responsible for the amazing increase in population during 1957.

This great range in insect population is correlated with the infestations at harvest shown in table 2. Brown rot, known to be associated with insect injuries to the fruit, made it impossible to accurately distinguish between wormy and stung fruit in 1953 and in 1957.

Wintering of Bees in Minnesota[1]

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ABSTRACT

Colonies of the University apiaries were divided into three groups of about equal strength. The colonies in one group were not packed, in another a light packing was applied, and those of the third group were heavily packed. Ample stores were left for wintering. The average mortality for the period of 1943–1944 to 1953–1954 was 18.4%, 6.4% and 2.9% for the non-packed, lightly packed and heavily packed groups, respectively. When the weak and queenless colonies were also counted, the losses were 34.3%, 15.2% and 10.5% for the above mentioned groups, respectively. The heavily packed group had, on an average, 25.0% and the lightly packed 19.2% more frames of brood in the spring than did the non-packed group.

Beekeeping is one of the oldest agricultural occupations. Many methods have been employed in the handling of bees and, in spite of the voluminous literature on the question of bee management, unanimous opinion is seldom reached even on some of the most important problems. The question of wintering, for instance, is one on which there is still considerable lack of agreement among both beekeepers and research workers.

Both scientists and practical beekeepers have been interested in the problem of wintering. Much of the literature was based on guesswork until Phillips & Demuth (1914) made a thorough study of the life of bees in winter cluster. These authors found that when the temperature in a colony of undisturbed broodless bees was above 57° F. and below 69° the bees were quiet and their temperature varies with the outer temperature. At a lower temperature the bees formed a compact cluster, and the temperature within it was raised by heat generated by the bees. (There is an outer shell several layers thick in the cluster of bees which preserves heat.) Subsequent experiments and observations by Wilson & Milum (1927), Sawin (1929), Corkins (1930), Corkins & Gilbert (1932) further enhanced our knowledge of the life of bees in winter. These studies had a very important bearing on the problem of wintering, because they gave an insight into the life of bees in the winter cluster.

Nevertheless, at present the beekeepers are still divided on the question of how best to protect their colonies during winter in order to help bees conserve heat and thus bring about smaller consumption of stores and better wintering.

Phillips & Demuth, (1914) on the basis of their experiments, recommended protecting the hives by packing them in wooden cases with an insulation of planer shavings several inches thick. Merill (1920, 1923) concurred with this and emphasized the importance of a windbreak, especially for colonies which have no other form of protection. The experiments conducted by King (1923) also pointed to the importance of good packing.

However, there is an increasing number of research workers who claim that neither heavy nor light packing is necessary. Wilson & Milum (1927) concluded from their observations that colonies winter well out-of-doors with no insulation or with from 1½ inches to 10 inches of insulation. Of hives which were protected from the prevailing winds by buildings or windbreaks, those without insulation seemed to do as well as those in packing cases. Milum (1930) found very negligible differences in the winter food consumption between packed and non-packed colonies. Farrar (1934, 1943, 1949, and 1952) stressed the importance of pollen for the wintering of bees. He demonstrated that brood-rearing in winter is a natural phenomenon and that those colonies which had enough

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stores of pollen and honey, and plenty of young bees and a good queen, wintered without packing and yielded just as good a crop as those wintered with such protection. He came to the conclusion that wintering in three stories is more satisfactory than in two stories. Packing in itself was of little or no value and heavy packing was worse than none. The locality was not much of a factor in wintering bees. There did not seem to be much difference in colony requirements, because the variations were as great between seasons in one locality as between localities. He stressed the importance which nosema, a protozoan disease of adult bees, plays on the outcome of wintering.

METHODS.—In order to test these two types of wintering, an experiment was conducted at the University of Minnesota Agricultural Experiment Station for a number of years. In the second half of August of each year the colonies were inspected and the number of frames of brood in each was ascertained. Then the colonies in each yard were divided into three groups in such a way that the colonies in each group had, on an average, about the same number of frames of brood. This was done in order to have in each group, colonies of approximately the same strength. Ample stores were left in each colony for wintering. All colonies had 1-inch insulite board on the top of the inner cover, with the bee escape hole left open. One group of colonies in each yard was left without packing. The colonies in the second group were lightly protected by either a corrugated paper case with a single building paper wrap or a double building paper wrap. The third group was well protected by either double corrugated paper cases or balsam wool wrap, both having one protective single building paper wrap besides, or half-inch insulite boards. Middle entrances were provided in all hives by placing strips of wooden shingles on the side walls of the first story. These entrances were contracted to about 3 inches in length. The lower entrances were completely closed.

The colonies in the Old Yard at the Fruit Breeding Farm at Excelsior, Minnesota, were located on a dry, very gradual south slope with no special windbreak nearby. The entrances of the hives faced south. The New Yard had a good windbreak from the south and west and there were low hills of apple orchards surrounding the apiary from the north and east. The location was not as well drained as the one on the Old Farm. The hive entrances faced south. The St. Paul Campus yard had a good windbreak from all sides except from the northnortheast. The hives were located on the northeast slope and the hive entrances faced northeast.

Before packing, all colonies were weighed. They were left undisturbed throughout the winter. An inspection in March was made to ascertain the number of dead colonies. Afterwards all the necessary manipulations were performed according to the advance of the active season. At the end of April, or the beginning of May, all colonies were weighed and later the number of frames of brood in each colony was counted.

RESULTS.—The results of the experiment for the winters of 1943–44 to 1953–54, inclusive, are presented in table 1. From the data presented it is evident that there was not much difference in the average food consumption of the colonies wintered in either group within each yard. However, consumption by the non-packed group in each yard was somewhat greater than in the packed groups. The consumption of stores in all three groups of colonies in the St. Paul Campus yard was the lowest. This was, possibly, due to a better wind protection in this yard. The average fall brood was about the same for the colonies in each group. However, the count of brood in the spring showed that a larger number of frames of brood

Table 1.—Results of packing versus not packing colonies of honey bees in separate yards in Minnesota during the winters of 1943–44 to 1953–54, inclusive.

YARD	NO. OF COLONIES	STORES CONSUMED (LB.)	FRAMES OF BROOD		NO. OF COLONIES LOST		
			Fall	Spring	Dead[a]	Weak and/or Queenless[a]	Total[a]
			Not-packed				
University Farm							
Old yard	99	60	5.4	4.1	21 (21.2)	10 (10.1)	31 (31.3)
New yard	83	61	5.1	3.6	16 (19.3)	17 (20.5)	33 (39.8)
St. Paul Campus yard	89	46	5.0	3.2	13 (14.6)	16 (18.0)	29 (32.6)
Total	271	56	5.2	3.6	50 (18.4)	43 (15.9)	93 (34.3)
			Lightly-packed				
University Farm							
Old yard	136	55	5.2	4.3	10 (7.4)	13 (9.6)	23 (17.0)
New yard	105	57	5.2	4.8	7 (6.7)	4 (3.8)	11 (10.5)
St. Paul Campus yard	121	42	4.9	3.9	6 (5.0)	15 (12.4)	21 (17.4)
Total	362	51	5.1	4.3	23 (6.4)	32 (8.8)	55 (15.2)
			Heavily-packed				
University Farm							
Old yard	102	56	5.0	4.7	3 (2.9)	4 (3.9)	7 (6.8)
New yard	69	54	5.4	4.9	3 (2.9)	4 (3.9)	7 (6.8)
St. Paul Campus yard	170	40	4.9	3.9	5 (2.9)	15 (8.8)	20 (11.7)
Total	341	50	5.1	4.5	10 (2.9)	26 (7.6)	36 (10.5)

[a] Percentages given in parentheses.

was present, on the average, in the packed colonies than in the non-packed group.

The most pronounced difference was in the number of colonies which were lost for the next year's production. The loss in dead colonies was the greatest in the non-packed groups in all yards, being three times as great as in the lightly-packed group and 5 to 7 times greater than in the heavily-packed group. It is apparent that there was also a considerable difference between the lightly-packed and the heavily-packed group. The average losses in dead colonies for all three yards was 18.4%, 6.4% and 2.9% for non-packed, lightly-packed and heavily-packed groups, respectively.

The cause of the death, in the vast majority of cases, was "cold starvation"—the bees were unable to move to the stores present in abundance in the hive, because of the protracted cold. Sometimes cold starvation was combined with dysentery. Cases of regular starvation were not counted. When the weak and queenless colonies were included in the wintering losses, the differences between the groups were considerable, being 34.3%, 15.2% and 10.5% for non-packed, lightly-packed and heavily-packed groups, respectively. The difference between the lightly- and the heavily-packed groups was not as great as between the non-packed and the packed groups. However, this difference was greater in the Old Yard than at the New Yard or the St. Paul Campus apiary. The difference in windbreak protection may have played a role here. The influence of nosema infection on wintering is being investigated at present.

The results of this experiment agree closely with a similar 13-year experiment conducted by Braun & Geiger (1955) in Manitoba. These workers found the losses in dead colonies 33.3%, 13.3% and 5.9% and the average spring brood count was 5.7, 7.0 and 8.7 frames for non-packed, lightly-packed and heavily-packed colonies, respectively.

These numbers closely correspond to those obtained in surveys made by Rea (1941) in New York (2.8% loss for heavy-packed and 50.8% for non-packed); Dunham (1941) in Ohio (10.9% for packed and 34.1% for non-packed); Haydak & Floyd (1955) in Minnesota (42.5%, 18.7% and 10.1% loss for non-packed, lightly-packed, and heavily-packed colonies, respectively). Thus it appears that under Minnesota conditions packing colonies helps the bees withstand the adversities of the winter weather better than if they are left without any protective covering.

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Chlorotic Fleck, an Eriophyid Mite Injury of Myrobalan Plum[1]

R. M. Gilmer and F. L. McEwen.[2] *New York State Agr. Expt. Sta., Geneva*

Abstract

Chlorotic fleck, a foliage abnormality of myrobalan plum (*Prunus cerasifera* Ehrh.), is induced by feeding of a toxicogenic eriophyid mite, *Vasates fockeui* (Nal. & Trt.). Although leaves of all ages are readily infested by the mites, symptoms of chlorotic fleck result only when the mites feed on immature leaves. Approximately 14 days elapse between the establishment of a mite infestation and the initial appearance of symptoms. Graft inoculations indicate that a virus is not involved. Excellent mite control and concomitant prevention of chlorotic fleck symptoms were obtained under field conditions with three applications of demeton, applied at approximately 2-week intervals.

A foliage abnormality of myrobalan plum (*Prunus cerasifera* Ehrh.), variously termed asteroid spot (Hildebrand 1945) or chlorotic fleck (Brase & Parker 1955), is common in New York fruit nurseries every year. Hildebrand (1945) observed that severely affected myrobalan seedlings were stunted and that the occurrence of chlorotic fleck in myrobalan stocks resulted in low bud-take or subnormal growth of Italian Prune (Fellenberg) budlings propagated on such stocks. Although Hildebrand (1945) did not demonstrate the cause of chlorotic fleck, his failure to reproduce the abnormality by bud-inoculation from affected seedlings to myrobalan or other *Prunus* species indicated that it was not virus-induced.

Brase & Parker (1955) found that the plum varieties Fellenberg, Stanley, and Abundance made less growth as budlings when propagated on myrobalan seedlings severely affected with chlorotic fleck than when propagated on seedlings only slightly affected. They suggested that chlorotic fleck might play some role in the initiation of constriction and decline of Stanley prune trees propagated on myrobalan stocks.

Occurrence and Symptomatology.—The prevalance of chlorotic fleck of myrobalan varies considerably from year to year in New York, but incidences of 90% or more in some fields are not unusual (Hildebrand 1945). Chlorotic fleck tends to be most prevalent and striking on yearling lining-out stocks and to diminish in intensity as the trees increase in size. In large trees, symptoms of chlorotic fleck, if present, occur on the foliage of suckers or watersprouts originating at or near the ground line.

Affected seedlings may develop symptoms of chlorotic fleck at any time during the growing season, but symptoms are most pronounced from late August to early October in New York. On some seedlings symptoms appear on half of the foliage or more, but on other seedlings, initially developing symptoms late in the growing season, symptoms may be confined to a single terminal.

Symptoms of chlorotic fleck consist of more or less well-defined circular chlorotic areas, ranging in size from mere pinpoints to spots 1 to 2 mm. in diameter, on the foliage of affected seedlings (figs. 1 and 2). The margins of the smaller flecks are often diffuse and indefinite, but the larger flecks usually possess well-defined margins and may occasionally show indications of one or two concentric rings. The number of flecks occurring on a single leaf may vary from 1 to 50 or more, and, if the flecks are sufficiently numerous the lamina of the affected leaf is wavy or slightly twisted about the longitudinal axis. Severely affected shoots are rosetted with many leaves that do not expand to normal size.

Symptoms may also occur on the bark of affected shoots. These spots are ovoid rather than circular in shape and tend to be larger than flecks occurring on the foliage.

Experimental Production of Symptoms.—From 1952 through 1955, on the hypothesis that chlorotic fleck might have a virus causation, numerous attempts were made to induce symptoms in several species of *Prunus* by bud-inoculation from affected myrobalan seedlings. None of these attempts were successful. Symptoms of chlorotic fleck failed to appear under greenhouse conditions in myrobalan seedlings that had previously shown severe symptoms in the field. Variation of greenhouse temperatures at which the seelings were grown, including temperatures of 80 to 85° F., 70° F., and a diurnal variation of 75° F. (days) and 55° F. (nights) failed to induce symptoms.

Attempts to isolate fungus or bacterial organisms from affected myrobalan foliage failed to result in the recovery of a possible pathogenic organism.

In August, 1956, examination of affected myrobalan seedlings in the field revealed a heavy infestation of an eriophyid mite, *Vasates fockeui* (Nal. & Trt.).[3] Attempts made in midSeptember to transfer and maintain cultures of this mite in the greenhouse failed, probably because the forms collected were deutogynes, a stage unable to reproduce until it has undergone hibernation (Keifer 1952).

In July, 1957, numerous transfers of individual mites to actively growing myrobalan seedlings were made with a camel's-hair brush. Transfers of this type resulted in injury and death to many of the mites, but two successful colonies were established in the greenhouse. Fourteen days after the date of transfer both of the myrobalan seedlings on which colonies had been established developed symptoms of chlorotic fleck in the foliage. Symptoms failed to develop on the leaves to which the actual transfers were initially made but appeared on the fourth and fifth leaves above the point of mite placement in one plant and on the fifth leaf above the point of placement in the second seedling infested.

Further transfers were made during August. At this time the mite population on myrobalan in the field was high and the distribution on the foliage was semi-gregarious. In making transfers areas of heavily infested leaf tissue were excised with a cork borer (7 mm. diameter)

[1] Journal Paper No. 1098, New York State Agricultural Experiment Station, Geneva, New York, December 3, 1957. Accepted for publication December 6, 1957.

[2] The authors are indebted to Professors K. D. Brase and S. E. Lienk for suggestions on some aspects of the problem.

[3] Identification by H. H. Keifer, California Department of Agriculture, Sacramento, Calif.

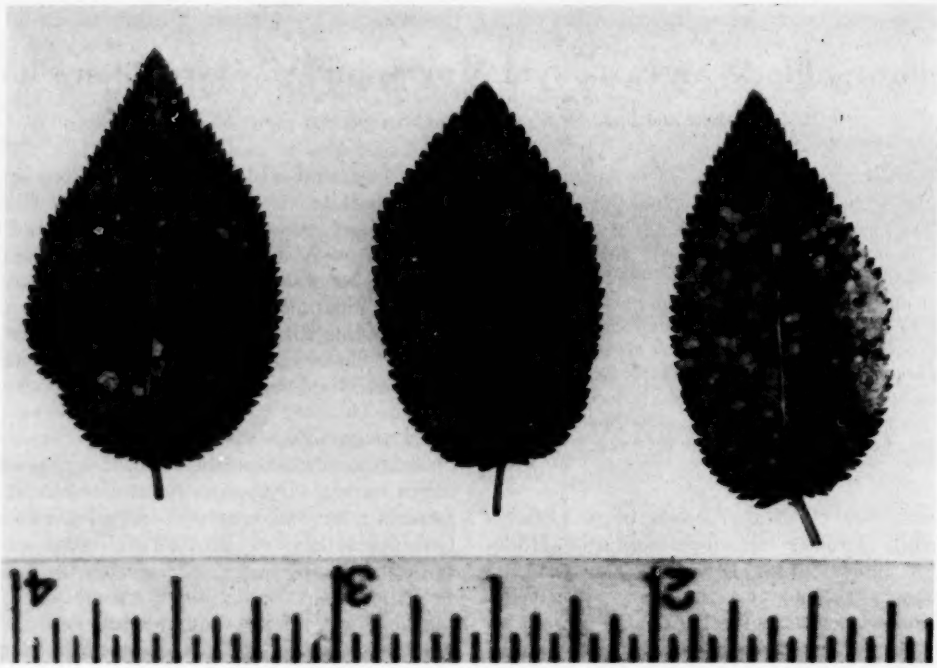

FIG. 1.—Symptoms of chlorotic fleck on young leaves of myrobalan artificially infested with *Vasates fockeui* (Nal. & Trt.).

and attached to the under surface of myrobalan leaves with ground wheat paste (Rex Brand Wallpaper Paste). This dried quickly and was non-toxic both to the mites and foliage. Within 24 hours the majority of the mites migrated from the gradually desiccating leaf disks and became established on the foliage.

Mite colonies thus established on mature leaves did not incite symptoms of chlorotic fleck on such leaves. Symptoms, however, again developed 13 to 33 days later on leaves that were immature or still in bud at the time of mite transfer. Leaves that developed symptoms of chlorotic fleck bore variable numbers of mites which had evidently migrated from the point of original placement on the mature foliage.

CONTROL.—Experiments for the specific control of *V. fockeui* in the field were not undertaken. In an experiment with demeton for control of potato leafhopper, *Empoasca fabae* (Harris), on myrobalan, three applications at approximate 2-week intervals from late June through early August resulted in complete control of these mites and of chlorotic fleck. Unsprayed controls in an adjacent field supported heavy mite populations and almost every seedling developed symptoms of chlorotic fleck.

DISCUSSION.—The data demonstrate the association of

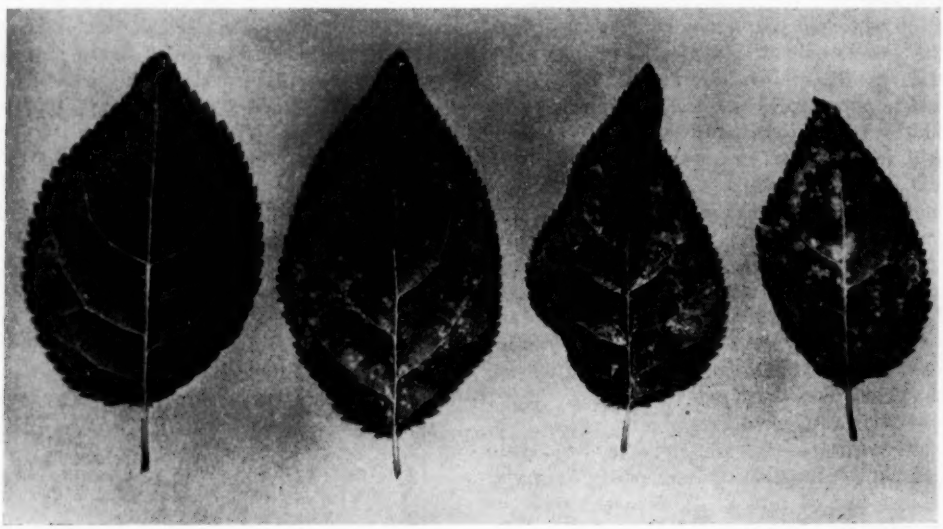

FIG. 2.—Symptoms of chlorotic fleck on young leaves of myrobalan naturally infested in the field with *Vasates fockeui* (Nal. & Trt.).

chlorotic fleck of myrobalan with infestations of the eriophyid mite, *V. fockeui*. The possibility of chlorotic fleck being a virus disease transmitted by *V. fockeui* appears to be conclusively excluded by the failure to induce chlorotic fleck symptoms either by bud-inoculation or by propagation from affected seedlings. The failure of symptoms to develop in the greenhouse in myrobalan seedlings that had previously shown severe symptoms in the field is also evidence against a virus causation of this abnormality.

The association of *V. fockeui* with a leaf-flecking of Bromptom plum has been observed in Germany (Schuch 1957) but the relationship between flecking and the mites was not definitely ascertained.

A similar foliage abnormality of peach, yellow spot, was found by Wilson & Cochran (1952) to result from feeding by a closely related mite, *Vasates cornutus* (Banks). According to these workers yellow spot symptoms were developed only when these mites fed on immature foliage, and, although feeding periods as short as 24 hours were sufficient to incite symptoms, these did not appear until 12 days after feeding had occurred. They concluded that yellow spot was not virus-induced, but that symptoms resulted from the injection of a toxic substance by the mites during feeding.

Symptoms of yellow spot of peach and chlorotic fleck of myrobalan are similar and the time lapses between actual mite feeding and the appearance of foliage symptoms are essentially the same. The close relationship of *V. cornutus* and *V. fockeui* (Keifer 1952) plus the above observed similarity with yellow spot of peach strongly indicate that chlorotic fleck of myrobalan is induced by the injection of a toxin by *V. fockeui*.

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Occurrence of a Toxic Non-Aldrin Residue in Carrots Grown on Aldrin-Treated Soil[1]

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ABSTRACT

Discrepancies in residues determined by several methods have been noted with soil applications of the insecticide aldrin to carrots. The present study was undertaken to determine the cause of the discrepancies among the three methods used: *Drosophila* bioassay, total chloride and phenyl azide. The experiments were designed to eliminate external contamination in the field and laboratory, to determine the efficiency of extraction of residues from carrots, and to rigidly test the methods of analysis.

Residues from direct bioassay of the carrots were significantly greater than those from chemical methods, which require preliminary extraction and chromatography. Direct bioassay and bioassay of unchromatographed Skellysolve-isopropanol extracts gave the same results. Bioassay residues of chromatographed extracts, however, were the same as the chemical residues, indicating that chromatography had removed a part of the toxic residue. All extractions with Skellysolve alone were less complete than those with Skellysolve-isopropanol.

Additional chromatographic analysis showed the presence of a second toxic constitutent. The sequence of chromatographic elution and the positive response of this compound to the reduction phenyl azide and total chloride methods but not to the phenyl azide method identified the compound as dieldrin. The biological evaluation of aldrin-dieldrin combinations proved the validity of equating the sum of the chemically determined aldrin and dieldrin values with the direct bioassay value.

The results of the quantitative determination of residues[4] in carrot plant material grown on soil treated with aldrin had caused concern since the bioassay of the macerated crop (Dewey 1955), a method based on toxicity to adult *Drosophila melanogaster*, Meig., indicated residues greater than those obtained by two chemical methods, aldrin-specific phenyl azide and semi-specific total chloride (O'Donnell *et al.* 1954). To determine the reason for this variance, an investigation was undertaken to eliminate the possibility of external contamination by other toxic materials, to rigidly test and evaluate the methods of analysis used; and, if the presence of a toxic non-aldrin residue was indicated, to attempt to isolate and identify it.

CARROT SAMPLES.—Two carrot samples, Red Cored Chantenay variety, were used in this study. One sample received a 4-pound per acre furrow-drench application of a formulation containing 99.3% aldrin in xylene with Tri-

[1] These data are a portion of the results of an investigation presented by the senior author in the dissertation for partial fulfillment of the requirements for the Ph.D. in the Graduate School at Cornell University. October, 1955.

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ton X-100 (Rohm & Haas Co., Philadelphia, Pa.) used as an emulsifying agent. The seed was sown on July 15, 1954, on New York sandy loam soil at Ithaca, N. Y. This plot, as far as could be ascertained, had never had any insecticide applied to it prior to this experiment. The formulation was applied at the time of seeding, using a small garden planter to which was attached a tank to hold the formulation. A ¼-inch copper tube running from this tank served to drench the seed as it was deposited in the furrow. Each replicate received approximately 500 ml. of the formulation. This carrot sample and its check (untreated) were harvested on October 16, 1954. The entire yield constituted the sample.

The second sample was supplied by Dr. A. C. Davis, Division of Entomology, New York State Experiment Station at Geneva and was taken from an experiment in which five insecticides (aldrin, dieldrin, chlordane, heptachlor and lindane) were being tested. The sample used in this study received a 12-pound per acre soil treatment of an aldrin formulation, containing a minimum of 97.4% aldrin. This plot was located at Geneva, N. Y. The soil type was Ontario loam. In 1950 the plot produced a crop of peas, in 1951 was sown to wheat, and in the 2 years preceding this experiment was used as pasture land. No insecticides were applied between 1950 and 1953. Previous use of soil insecticides is highly improbable. At the time of seed bed preparation, the plot received 100 pounds of nitrate and 500 pounds of 5:10:10 fertilizer per acre. The aldrin formulation was applied as a soil treatment on May 18, 1954, using a low pressure sprayer with a 12-foot boom. It was applied at the rate of 101 gallons per acre using a pressure of 60 p.s.i. and was harrowed into the soil immediately following the application. The seed was sown on June 10, 1954, and the same was harvested along with the check on October 18, 1954.

THE EFFECT OF EXTERNAL CONTAMINATION.—In any residue determination a real problem is posed by the possibility of external contamination of the crop material prior to submission for analysis. In this study it was essential that the effects of external factors be determined if present, and if possible be excluded as a contributing source of variance.

The use of check or untreated plant material along with the treated samples is a standard practice in residue studies. If the check exhibits a residue it may indicate persistence in the soil of toxic materials from a previous application, careless application of the insecticide, transfer of the toxicant from treated to untreated crop materials by some factor such as drainage, or cultural practices, contamination of the sample in the field at or following harvest, or contamination of the sample in the laboratory prior to analysis.

Since the locations of the treated and untreated carrot samples in the plots were randomized it was reasonable to suppose that the untreated and treated samples would be equally susceptible to careless application of the formulation, and other contamination in the field. In the laboratory the untreated and treated samples were subjected to the same pre-analytical treatment, and as above would be expected to indicate any contamination from this source. The untreated samples were assayed by bioassay of the macerated crop, the method which had indicated the highest residues with the treated carrot samples. The results of this assay are shown in table 1. From these results it was fairly obvious that the untreated carrot samples contained no toxic residue. Therefore, contamination from the above factors was discounted.

One other external factor had to be considered—the presence of toxic material other than aldrin in the formulation. In the carrot sample receiving 4 pounds of aldrin per acre this possibility would seem unlikely since this aldrin was labelled to be of 99.3% purity. Corroboration of a high degree of purity was also indicated by our own studies. The exact purity of the aldrin used in the 12-pound per acre formulation was not known, but the label indicated a minimum of 97.4% purity. The quantitative and qualitative results (see tables 2, 3 and 5) obtained from the samples of carrots treated with 12- or 4-pound dosages of aldrin per acre were similar, hence the 12-pound aldrin formulation was considered comparable to the 4-pound treatment in regard to the lack of toxic impurities. From these considerations, it does not appear that external contamination contributed in any way to the variance in the results.

COMPARISON OF ANALYTICAL METHODS.—The chemical methods (O'Donnell *et al.* 1954) call for the use of Attasol (Attapulgus Clay Co., Philadelphia, Pa.) chromatographed extracts of the plant material, while the bioassay method (Dewey 1955) is performed directly on macerated crop. It was desirable to evaluate raw and chromatographed extracts by bioassay to determine efficiency of extraction and whether or not a toxic material was being removed by the chromatographic clean-up procedure. Therefore, the following technique was developed.

For the bioassay of chromatographed and unchromatographed extracts, 0.5-ml. aliquots of the extracts (equivalent to 0.25 gm. of the whole crop) were introduced into the residue exposure chambers (#9800 Corning 25×200 mm. Pyrex test tubes) using a 1.0-ml. graduated pipette. The solvent was allowed to evaporate, while the tubes were slowly rotated in a horizontal position. This allowed an even deposit of the toxicant to be distributed on the inner surface of the residue exposure chamber. The chamber was closed with a cotton plug, which held a No. 2. 1½-inch dental cotton roll. This dental cotton roll had been soaked in a water solution of 5% "bee candy" and 2.5% Brewer's yeast, and so provided food and water for the test insects. A standard curve of the appropriate (chromatographed or unchromatographed) untreated carrot extract fortified with 3.2 to 0.025 p.p.m. aldrin in terms of the original sample was prepared. One-half-ml. aliquots of the desired dilutions were pipetted into the residue exposure chambers thus providing a range of 0.8

Table 1.—Apparent aldrin residue obtained by bioassay of the macerated untreated carrot samples submitted for analysis with samples treated with the 4 or 12 pounds of aldrin per acre.

	PARTS PER MILLION			
	Aliquot			
CHECK SAMPLE FOR	1	2	3	Mean
4 pounds per acre	0.000	—	—	0.000
12 pounds per acre	0.000	0.010	0.000	0.003

Table 2.—Apparent aldrin residues obtained by analysis of carrot sample treated with 12 pounds of aldrin per acre.

Method of Analysis	Extraction[a]	Parts per Million	
		Mean[b]	Standard Deviation
Bioassay			
Macerated Crop	None	0.299	±0.004
Extract	SI	.291	±0.031
Extract	S	.149	±0.024
Extract chromatographed	SI	.141	±0.004
Extract chromatographed	S	.072	±0.027
Chemical[c]			
Phenyl azide	SI	0.169	±0.031
Total chloride	SI	.139	±0.003
Phenyl azide	S	.030	±0.027
Total chloride	S	.015	±0.008

[a] S=Skellysolve B; SI=Skellysolve B-isopropanol.
[b] Average of three determinations.
[c] Corrected for average recoveries.

to 0.00625 micrograms aldrin per chamber. Mortality was observed at 8-, 16-, 24-, 32- and 40-hour intervals. The mortality nearest the 50% level, usually at the 16-hour interval, was used in the determination of the apparent aldrin residues. The mortality data obtained by the exposure of 15 replicates of 20 insects each to check and treatment aliquots of the extracted carrot samples were referred to the standard curve.

Two methods of extraction of plant material for aldrin residues have been described by O'Donnell *et al.* (1954). The use of Skellysolve B (Skelly Oil Co., Tulsa, Okla.) was recommended for crops low in water content, and Skellysolve B-isopropanol (2/1 v/v) was recommended for crops high in water content. These two extraction procedures were compared for carrot plant material on all of the methods except bioassay of the macerated crop. Two volumes of solvent were used to extract one weight of macerated carrot tissue.

Upon completion of all assays for residues in the carrots treated with 12 pounds of aldrin, the data were tabulated (table 2) and tested for homogenity using Bartlett's test (Snedecor 1946) with 8 degrees of freedom. A chi square of 1.733 was obtained which was well within the value of 15.507 given at the 5% level thus indicating safe use of the analysis of variance as originally planned. The analysis of variance showed that the treatments were significantly different at the 1% level (F=73.88). Dunnett's (1955) method of comparing several treatments with a control or standard was then employed using the error variance obtained in the analysis of variance with 27 degrees of freedom. The upper and lower limits for each of the analytical methods using the phenyl azide method of the Skellysolve isopropanol extracts as the standard are given in table 4, with P=95%. The allowance for two sided limits is ±0.049. The following conclusions were drawn:

Table 3.—Apparent aldrin residues obtained by analysis of carrot sample treated with 4 pounds of aldrin per acre.

Method of Analysis	Extraction[a]	Parts per Million
Bioassay		
Macerated crop	None	0.304
Extract chromatographed	SI	0.189
Chemical		
Phenyl azide	SI	0.160
Total chloride	SI	0.170

[a] SI=Skellysolve B-isopropanol.

Table 4.—Apparent aldrin residues of macerated crop and Skellysolve B-isopropanol extracts of carrot sample treated with 12 pounds of aldrin per acre with confidence limits, using the phenyl azide chemical method as a standard of comparison.

Method of Analysis	Parts per Million	
	Mean	Confidence Limits P=95%
Bioassay		
Macerated crop	0.299	0.250–0.348
Extract	.291	0.242–0.340
Extract chromatographed	.141	0.092–0.190
Chemical		
Phenyl azide	0.169	0.120–0.218
Total chloride	.139	0.090–0.188

1. The whole macerated crop residue exceeds the phenyl azide method by an amount of 0.081 p.p.m. to 0.179 p.p.m. and the bioassay of the raw extract was found to exceed the phenyl azide chemical method by 0.073 p.p.m. to 0.171 p.p.m. Thus, both of these bioassay methods overlapped each other and could not be considered different from one another. Both are significantly greater, however, than the phenyl azide chemical method.

2. The bioassay of the chromatographed residues differed from the standard by an amount of 0.077 p.p.m. less to 0.021 p.p.m. greater, and were in the same range as the semi-specific total chloride chemical method which differed from the standard by an amount of 0.079 p.p.m. less to .019 p.p.m. greater. All chromatographed extracts fell in the same area of efficiency and were inferior to the assay of the macerated whole crop or the raw extract using bioassay.

These results were further substantiated by the 4-pound per acre treatment (table 3) which was based on single aliquots due to the small size of the sample and, therefore, not treated statistically.

3. The two methods of extraction, Skellysolve B alone and the Skellysolve B-isopropanol mixture, were compared by subjecting all the data available from the 12-pound treatment (table 2) to the "T" test using the error variance obtained in the analysis of variance. The mean for the Skellysolve extract was 0.066 p.p.m. of aldrin as compared with 0.1182 p.p.m. of the Skellysolve-isopropanol extracts. This difference is highly significant as a value of 287.7 for "t" was obtained in comparison with 3.055 required for significance at 1%. Hence the straight Skellysolve extraction of these carrot residues was inadequate.

THE METABOLITE EXPERIMENT.—From the above comparison of methods, it was obvious that chromatography removed a significant fraction of toxic material present in the whole crop and in the unchromatographed crop extract. Since satisfactory recoveries were obtained when fortified extracts were chromatographed and ana-

Table 5.—Aldrin and dieldrin residues in magnesium oxide chromatographed Skellysolve B-Isopropanol extracts of treated carrot samples.

Method of Analysis	Residue (p.p.m.) Aldrin (1st 100-ml. Fraction)	Dieldrin (3rd 100-ml. Fraction)	Total Aldrin and Dieldrin
Samples treated with 12 lb. aldrin per acre			
Phenyl azide	0.187	—[a]	0.321
Reduction-phenyl azide	—[a]	0.134	
Total chloride	0.138	0.158	0.296
Bioassay	0.158	0.140	0.298
Samples treated with 4 lb. aldrin per acre			
Phenyl azide	0.160	—[a]	0.299
Reduction-phenyl azide	—[a]	0.139	
Total chloride	0.170	0.143	0.313
Bioassay	0.189	0.101	0.290

[a] Values within the limits of the method for zero residue.

lyzed by the above methods, any unaccounted loss of aldrin in the chromatographic step was ruled out. A non-aldrin toxic material must, therefore, be present; initial speculation suggested dieldrin, the epoxy derivative of aldrin, as a possibility.

Negative evidence for this hypothesis is supplied by the observation of O'Donnell *et al.* (1954) that the Attasol chromatography step removes dieldrin from the aldrin fraction. Furthermore, dieldrin does not interfere in the phenyl azide method for aldrin. To obtain positive evidence for the presence of dieldrin, it was decided to compare the carrot residues with known aldrin-dieldrin mixtures in regard to: 1) their chromatographic behavior on the magnesium oxide-Cellite column used in the dieldrin analysis of O'Donnell *et al.* (1955), 2) their reactivity under the conditions of the dieldrin-specific reduction-phenyl azide method (O'Donnell *et al.* 1955), and 3) the balancing of the residue totals by the appropriate combination of the different methods.

Preliminary experiments were conducted to determine the distribution of aldrin and dieldrin in the eluates of fortified check carrot extracts (Skellysolve B-isopropanol) when chromatographed on a magnesium oxide-Cellite (Johns-Manville Products Corp., New York, N. Y.) (2/1 w/w) column with Skellysolve B as eluting fluid. When elution fractions were analyzed by total chloride and by bioassay, dieldrin appeared in the third 100-ml. fraction as expected (O'Donnell *et al.* 1955) and aldrin appeared in the first 100-ml. fraction. The non-interference of aldrin in the reduction-phenyl azide method for dieldrin was corroborated by direct analysis of a series of aldrin-dieldrin mixtures at the expected residue levels.

Two hundred-ml. aliquots of the Skellysolve B-isopropanol extracts representing 100 gm. of the treated carrots samples were chromatographed as above. With the 12-pound per acre sample, twelve 100-ml. fractions were taken and analysed by the total chloride, the bioassay, the phenyl azide and the reduction-phenyl azide methods. No significant residues by any of these methods were found in any of the fractions except 1 and 3. The results (table 5) for fractions 1 and 3 for both carrot samples showed satisfactory agreement among the analyses for each fraction.

Table 6.—Total residues in terms of aldrin in carrot samples.

Method of Analysis	Aldrin (p.p.m.) In 1st 100-ml. Fractions	Equivalents in 3rd 100-ml. Fractions	Total Equivalents
Sample treated with 12 lb. per acre			
Phenyl azide	0.187	—[a]	0.285
Reduction-phenyl azide	—[a]	0.098	
Total chloride	0.138	0.115	0.253
Bioassay	0.158	0.102	0.260
Bioassay	Unchromatographed extract		0.291
Bioassay	Macerated Crop		0.299
Sample treated with 4 lb. per acre			
Phenyl azide	0.160	—[a]	0.261
Reduction-phenyl azide	—[a]	0.101	
Total chloride	0.170	0.104	0.274
Bioassay	0.189	0.074	0.263
Bioassay	Macerated crop		0.304

[a] Values within the limits of the method for zero residue.

The sums of these residues from fractions 1 and 3 were then compared with the total residues obtained by direct bioassay (table 2 macerated crop and raw extract). Since the total residues by direct bioassay were expressed in terms of aldrin, it was necessary to convert the summed residues from the chromatographed extracts to aldrin equivalents according to the concepts of the joint action of insecticides. Using the method of Wadley (1945, 1949) similar joint effect was demonstrated from the dosage-mortality curves for aldrin, dieldrin, and a mixture of aldrin and dieldrin, hence one toxicant can be substituted for the other at a constant rate. Using the raw carrot extract, 0.73 unit of aldrin was found to be equivalent to 1 unit of dieldrin. Applying this equivalence factor to the dieldrin (fraction 3) residues in table 5, and summing with the aldrin (fraction 1) residues in the same table, the total residues in the chromatographed extracts are now expressed in terms of aldrin (table 6). Thus, the toxicity obtained in the direct bioassays appears to be accounted for in terms of the aldrin and the dieldrin found present in the chromatographed extracts.

The evidence presented strongly suggests that aldrin was converted to dieldrin either in the carrot plant material and/or in the soil in which the carrot was grown. Since biological epoxidations in mammals have already been observed with heptachlor (Radomski & Davidow 1953) and, more recently, with aldrin (Bann *et al.* 1955, Winteringham & Barnes 1955) a similar metabolism by plant tissues or soil microorganisms is not unreasonable. Further work should be directed toward identifying the source and nature of this epoxidizing system.

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Artificial Dissemination of Entomophthorous Fungi Pathogenic to the Spotted Alfalfa Aphid in California[1]

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ABSTRACT

Following evaluation of methods that could be utilized in the dissemination of fungi pathogenic to the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), a program was developed to produce *Entomophthora exitialis* Hall & Dunn, *E. virulenta* H. & D., and *E. oronata* (Cost.), on selected media in small sealed containers for shipment to counties within the State of California requesting fungi. During 1956, a total of 1,754 culture units were sent to nine counties in the San Joaquin and Sacramento valleys for placement in field plots.

Less than two years after the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), made its appearance in California in 1954, it was found to be attacked by several species of pathogenic entomophthorous fungi (Hall & Dietrick 1955, Hall & Dunn 1957a). Close observation of many fields in the coastal and low desert areas of southern California disclosed numerous fungus epizootics resulting in effective control of high populations of the pest. When these continuous surveys indicated that the fungi were not spreading to other parts of the State as fast as the aphids were, attempts were made during the summer of 1956 to aid natural spread by a program of artificial dissemination of the fungi.

METHODS CONSIDERED FOR DISSEMINATION OF THE FUNGI.—Limitations of time and personnel necessitated careful selection of the most economical, yet effective, method that could be employed in the distribution of the fungi. The following four methods were considered.

1. *Transfer of cut alfalfa bearing dead and living infected aphids from a field where an epizootic was in progress to fields having a disease-free aphid population.*—This method was tried experimentally with considerable success in the Imperial Valley of California, where the Agricultural Commissioner sanctioned the movement of fresh hay between fields in Imperial County. This is considered an excellent method for distribution of the fungi, especially in a localized area, but was ruled out for use in a statewide program in 1956 because the State of California Bureau of Plant Quarantine did not permit this type of alfalfa movement from counties in southern California to other parts of the State.

2. *Transfer of living infected aphids from a field where an epizootic was in progress to fields having disease-free aphid populations.*—The diseased aphids join the field population on the alfalfa, succumb from the infection, and become a source of infection for adjacent aphids. This method is essentially a manipulation of a phenomenon which commonly occurs in nature, since distribution of the fungi by natural means from field to field generally is accomplished by the flying-in of infected winged aphids. In the one attempt made, the method proved to be workable, but it was found to have important limitations. As in the transfer of alfalfa, the movement of infected aphids may be excellent for fungus distribution within an isolated area, but because of the difficulty of obtaining large numbers of aphids free from other alfalfa pests and the danger of transferring other pest insects along with the aphids, plus the uncertainty of the availability of suitable infected populations when needed, it was considered unsatisfactory for a program of distribution on a statewide basis.

3. *Production of resistant and other stages of the various fungi on artificial media in the laboratory, for application in sprays or dusts to aphid-infested fields.*—Some preliminary experimentation was conducted in cooperation with representatives of Nutrilite Products, Inc., Buena Park, California, at their Hemet and Lakeview (Riverside County) farms. Two tests were made by spray application of resting spores and vegetative stages of one of the most virulent fungi. Neither test resulted in establishment of the fungus. On the basis of these tests and because of limited time for further investigation, this method was considered impractical for immediate use.

4. *Production of cultures of pathogenic fungi for placement in fields having disease-free aphid populations.*—This method is similar in many respects to the well-tried colonization of introduced species of insect parasites.

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Cultures of the organisms grown on artificial media are placed in fields in such a manner that forcibly discharged conidial spores will land on susceptible aphid hosts. The conidia readily adhere to their host and are a source of infection of any aphid on which they land. Once the fungi are successfully introduced, they tend to spread from the point of establishment. This spread usually is gradual and depends on the abundance of the aphid host in successive generations as well as on favorable humidity and temperature in the microenvironment.

Although this method has been reviewed by other workers in past efforts to utilize entomophthorous fungi, it has not been tried often because of the general feeling that the organisms tend to lose virulence when cultured on artificial media with the result that few insects are infected in field inoculation attempts. Consideration was given to this possible drawback with regard to the present problem, and since this was the only method feasible for distribution of the organisms because of existing state quarantine regulations, it was thought worth a try. In the light of numerous field observations of the start of fungus epizootics, even if only a few spotted alfalfa aphids were infected from the cultures, successful establishment of the pathogenic fungi could be assured by the subsequent rapid spread of the infection from the points of introduction.

The species of entomophthorous fungi pathogenic to the spotted alfalfa aphid have been found to grow and sporulate readily on a variety of inexpensive media. In a series of pilot tests, cultures grown on artificial media were inverted over aphid-infested alfalfa plants in the Antelope Valley of Los Angeles County and near Cawelo in Kern County, two areas where the organisms had never been observed previously. The appearance of fungus-killed aphids immediately under some of the culture cartons while not noted elsewhere in the fields indicated that the fungus diseases could be transmitted to the aphid populations in the fields by this method. On the basis of these tests, it was decided that this method of dissemination of the fungi was the most practical for use by the insect pathology unit at Riverside, in an effort to establish the fungi in areas infested with the spotted alfalfa aphid in central and northern California during the summer of 1956.

PRODUCTION AND DISTRIBUTION OF INFECTIVE MATERIAL.—Three species of entomophthorous fungi isolated from the spotted alfalfa aphid were selected for production and dissemination in this program. Two of these species, *Entomophthora exitialis* Hall & Dunn (1957b) and *Entomophthora virulenta* Hall & Dunn (1957b) are highly pathogenic to the aphid. The third species, *Entomophthora coronata* (Cost.) Kevorkian (1937), apparently does not occur commonly in nature (see Hall & Dunn 1957b), but is the most striking producer of conidial spores of the fungi that attack the aphid and hence was considered worth trying on a wide scale.

The three fungus species were cultivated on a variety of media in a search for a material which would give rapid growth and good conidial spore production. Sabouraud Dextrose Agar (Difco) was found to be the most suitable for *Entomophthora coronata* and *E. virulenta*. The most pathogenic species, *E. exitialis*, was found to be poorly adapted to growing on artificial media despite many attempts with different combinations to get good growth and abundant conidial production. A medium of Sabouraud Dextrose Agar fortified with 5% triturated breakfast cereal (Special "K", Kellogg Co., Inc., Battle Creek, Michigan) was finally selected for use in the production of *E. exitialis*. This medium was easily and cheaply prepared in quantity and gave fair growth and good production of conidial spores.

The need for disposable units suitable for cultivation, shipment, and inverted field placement of the fungus cultures resulted in the selection of ½-pint waxed food containers with tight-fitting solid covers. Each unit created an environment in which a species of entomophthorous fungus would grow profusely on an artificial medium, forming many conidial spores which would not be discharged forcibly until light and a lower humidity were encountered when the cover was removed. Because of the necessity for inverting the cultures in the field, the bond between the medium and the carton was strengthened by using 3% agar to prevent curling or separation. Tests in the field indicated that the 3% agar medium remained intact in the open inverted cartons for a minimum of 5 days, after which time, under normal field conditions, the medium hardened to a tough crust and the fungus culture was no longer effective.

The culture cartons were prepared by injecting 25-ml. amounts of melted agar medium into each covered container, utilizing a 50-ml. syringe with a 1½-inch, 15-gauge needle. The syringe was connected to a 3,000-ml. flask of heated agar culture medium by rubber tubing and a Beckton Dickinson automatic double valve which permitted continuous filling and emptying of the syringe, thereby greatly accelerating the preparation procedures. The hole in the carton cover made by the hypodermic needle was covered with a small piece of masking tape. The cartons containing the medium were sterilized in an autoclave at 15 p.s.i. for 10 minutes.

Attempts to utilize the syringe-and-needle method for transfer of a slurry of fungus inoculum to each carton gave unsatisfactory results. Since time did not permit additional experimentation to develop a workable procedure for rapid inoculation, the transfers were made by the standard loop method, which involved the careful removal and replacement of the fragile paper cover of each carton. To maintain high humidity within the units during the period between inoculation and placement in the field, the cartons were sealed by dipping in melted paraffin.

Usually the units were prepared and inoculated on Monday of each week and the cultures were incubated at room temperature (about 25° C.) for 4 days. On Friday, random units were examined to determine the condition of the cultures, and the cartons were packed in cardboard containers, along with suitable wire holders to support each culture unit in an inverted position in the field, for shipment to the agricultural commissioners of recipient counties. Taking advantage of the weekend period for part of the transit time, the cultures usually arrived at their destination about 7 to 10 days after inoculation and were placed in the field immediately or refrigerated until such distribution could be made.

The number of cultures to be placed in each release site was left to the discretion of the county agricultural commissioner receiving the material and was dependent upon the extent of the aphid infestations and the number of release sites available. It was suggested that at least two cultures of each fungus species be placed in each release site to enhance the chances of the three species becoming established. The following recommendations were made for consideration in the selection of release sites for the fungi.

1. *Humidity.*—Since adequate moisture is required for successful infection of the aphids, only fields under standard irrigation practices (either flood or sprinkler) should be selected for inoculation. A moist area adjacent to a stand-pipe would be an excellent site.

2. *Aphid supply.*—Since the fungi are dependent upon the aphid for reproduction, a moderate spotted alfalfa aphid population should be on the plants in the release sites.

3. *Height of hay.*—Alfalfa in the plots should be at least 16 to 20 inches in height to shade the culture and to provide the necessary cover for the development and maintenance of relative humidities high enough to activate the fungi.

4. *Cutting.*—Plots should be left uncut for at least one cutting to permit the establishment and spread of the pathogenic organisms.

5. *Insecticidal treatment.*—To insure an aphid supply for the fungi, no insecticides should be applied to the plot during the inoculation attempt.

6. *Size of plot.*—Size is of little importance if the other requirements are met. If possible, the same plot used for release of insect parasites may be utilized for inoculation with the fungi.

RESULTS OF DISSEMINATION PROGRAM.—A total of nine counties in the San Joaquin and Sacramento valleys entered into this cooperative program and received one or more shipments of fungus cultures (see table 1). A preliminary report giving information on this program has been made by the authors (Hall & Dunn 1957a), integrating information from various sources throughout California.

It is difficult to evaluate the results of a program of artificial dissemination of pathogenic fungi, since the evidence at best is circumstantial and natural spread of the organisms must be considered as a possibility in every instance of establishment. This is especially true in the case of Kings County, since in neighboring Tulare County the fungi effected natural control of the spotted alfalfa aphid during the summer of 1956, and natural spread of the organisms into Kings County carried by migrating winged aphids was quite possible.

The release of fungus material to other workers in the various counties for the actual introductions and subsequent determinations of establishment of the organisms in the release sites created a problem in the evaluation of results of the whole program since none of the county personnel were familiar with the fungi and their actions against the spotted alfalfa aphid in the field. In most instances, the county workers were able to detect the presence of the pathogenic fungi only when great numbers of aphids were succumbing. Since surveys for the most part were on an irregular basis, the chances were great of missing the appearance of diseased aphids in the fields, regardless of the degree of fungus activity. With the cutting cycle and the additional variability of the aphid populations which often tend to remain at very low levels for some time following fungus attacks, it is understandable why little information was received on the establishment of the fungi in the inoculated areas within a reasonable time after the cultures were placed in the fields.

Table 1.—Shipments of fungus cultures (*Entomophthora* spp.) to California counties, June 25 to October 31, 1956 for control of *Therioaphis maculata.*

COUNTY	*E. coronata*	*E. exitialis*	*E. virulenta*	TOTAL
Amador	21	21	21	63
Glenn	19	10	19	48
Kings	124	94	124	342
Madera	48	48	48	144
Merced	58	—	61	119
Sacramento	80	60	80	220
San Joaquin	92	80	92	264
Solano	104	78	104	286
Tehama	100	68	100	268
Totals	646	459	649	1,754

In the only survey of release sites conducted by the authors, some of the fields inoculated with the fungi in Kings County were examined about 2 weeks after cultures were shipped to the area. In one of the best release locations where the aphid population was heavy, a small number of fungus-killed aphids was found in the vicinity of the culture cartons. At the same time, no diseased aphids were observed elsewhere in the field, indicating a possibility that the initial infection had been successful.

More favorable developments to support the success of the artificial distribution program occurred in Tehama County, about 300 miles north of the line of advance of the naturally spreading fungi. This area was the first to show aphid buildups in the summer of 1956 and the first to receive shipments of fungus cultures. Although Tehama County workers did not observe establishment in the release sites, the first signs of outbreaks of disease were noted in September and fungus-caused epizootics soon developed in many fields. The receipt of specimens of dead spotted alfalfa aphids from Tehama County revealed that the fungus responsible for the heavy aphid mortality was *Entomophthora exitialis*. The other species of fungi were not recovered. The Glenn County distribution program, which was undertaken late in the season, was limited to one shipment of 48 cultures because outbreaks of disease were noted shortly after the release was made. Both *E. exitialis* and *E. coronata* were observed in specimens received from Glenn County.

The fungus *E. exitialis* was observed in specimens of dead aphids received from Madera County after several shipments of fungus cultures were placed in the fields. On the other hand, no diseased specimens or reports of establishment of any of the three species of fungi were received from Amador, Merced, Sacramento, San Joaquin, or Solano counties, each of which received cultures for field placement.

It is interesting that of the two species of fungi displaying known marked pathogenicity for the spotted al-

falfa aphid, *E. exitialis* and *E. virulenta*, only *E. exitialis* has spread by natural or artificial means and become established in parts of California north of Tulare County. *E. virulenta*, which is widespread in southern California, has been observed on only a few occasions in Tulare County, where it appeared to be successfully established in 1955, and has yet to be noted elsewhere in central and northern California.

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The Thermal Death Points of Several Species of Insects[1]

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ABSTRACT

Investigations were made to determine the mortality of various insects when exposed to conditions simulating those found in closed uninsulated aircraft parked in the sun and in unrefrigerated compartments of supersonic aircraft. Fifteen species, including the American dog tick (*Dermacentor variabilis* (Say)), were exposed in a forced-draft electric oven for 15 to 60 minutes to temperatures ranging from 40° to 60° C. The various species differed widely in resistance to heat, but all were dead when exposed for 15 minutes at 60° C. These findings have quarantine significance, especially in supersonic aircraft where heat is generated by friction on the skin.

Each year thousands of aircraft are inspected to prevent the spread of hitchhiking insects dangerous to agriculture and public health. During recent years much research has been conducted to develop suitable disinsectization treatments. Previous studies have shown that the cold, reduced pressure, and low humidity in the stratosphere where jet aircraft normally operate kill insects in unheated compartments (Knipling *et al.* 1957, Sullivan *et al.* 1958). It is of interest to know what happens to any insects that survive such conditions, and are subsequently exposed to high temperatures which are often reached when the aircraft is parked and closed.

In the United States, military and commercial aircraft total 45,384 (Witz 1957, Lewis 1957). Of this number 43,226 are military owned, 1,382 are domestic carriers, and 776 are in international service. Nearly all the military aircraft are uninsulated except for the pilot's compartment, and when they are closed and parked in the sun the inside temperatures often become very high. This is true during the summer months in temperate regions and throughout the year in the tropics. The Air Force (Grim 1953) found that, when B-47's are exposed to desert environment, temperatures within compartments may exceed 71° C., and that 50° is not uncommon. The highest temperature recorded was 97° in the pilot's cockpit enclosed by a transparent area.

In supersonic aircraft another source of high temperatures arises. Friction on the outer surface of the plane may produce enough heat to kill insects in unrefrigerated sections, especially when the plane is flying at about twice the speed of sound.

PROCEDURE.—In order to obtain additional information on the fate of insects and ticks in environments simulating those likely to be encountered in aircraft tests were run at Beltsville, Md. in the summer of 1957 to determine the thermal death points of several species. Fifteen species were used in these tests. The Mexican bean beetle (larvae and adults), the Japanese beetle, the American dog tick, several species of grasshoppers, and the Colorado potato beetle were collected in the field, and the northern house mosquito, the common malaria mosquito, the yellow-fever mosquito, the house fly, and the confused flour beetle were laboratory-reared.

The confused flour beetles and the American dog ticks were placed in glass vials with cloth tops and the other insects in cylindrical wire-screen cages. A nearly constant number of each species of laboratory-reared insects was used per test—yellow-fever mosquitoes nearly 100, northern house mosquitoes 50, house flies 30, flour beetles 25, and common malaria mosquitoes approximately 10. Of the field-collected species 10 to 15 insects were generally used per test, but there was often a wide variation from the average due to difficulty in obtaining the desired number at some times. The field insects were collected each day for the tests.

The insects were exposed for various periods from 15 to 60 minutes to temperatures from 40° to 60° C. in a forced-draft electric oven which gave equal distribution of heat. A sensitive control dial on the oven made it easy to obtain accurate readings. The oven was situated in an air-conditioned room that insured room temperatures of 23° to 27° C. By means of wet- and dry-bulb thermometers, the relative humidity and dew point were recorded each day. The humidity range in the room was 32% to 50%, and in the oven, as calculated from the oven temperatures and dew points, 6% to 21%. At the end of each test period the insects were fed and kept for a 24-hour observation period in an outdoor insectary where temperatures ranged from 10° to 35° C. Approximately 900 tests were conducted in this manner.

RESULTS.—The mortalities of all test insects after the various exposures are given in table 1. After all exposure periods the northern house mosquitoes and common malaria mosquitoes showed 100% mortality at 45° C.; yellow-fever mosquitoes and Mexican bean beetle larvae at 50°; and Mexican bean beetle adults, house flies, Japanese beetles, confused flour beetles, and American dog ticks at 55°. The grasshoppers and Colorado potato beetles were the hardest to kill, 60° being required for

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Table 1.—Effect of heat on the mortality of 14 species of insects and the American dog tick.[a]

Species	Minutes Exposure	Per Cent Mortality 24 Hours After Exposure at—				
		40° C.	45° C.	50° C.	55° C.	60° C.
Northern house mosquito (*Culex pipiens* L.)	15	14 ± 2	100	100	—	—
	30	96 ± 2	100	100	—	—
	45	100	100	100	—	—
	60	100	100	100	—	—
Common malaria mosquito (*Anopheles quadrimaculatus* Say)	15	16 ± 8	100	100	—	—
	30	48 ±11	100	100	—	—
	45	55 ±10	100	—	—	—
	60	71 ±17	100	—	—	—
Yellow fever mosquito (*Aedes aegypti* (L.))	15	0.5± 0.5	83± 8	100	—	—
	30	1 ± 1	100	100	—	—
	45	8 ± 3	100	100	—	—
	60	24 ± 7	100	100	—	—
Mexican bean beetle (*Epilachna varivestis* Muls.)	15	0	3± 3	100	100	—
	30	0	88± 6	100	100	—
	45	0	100	100	100	—
	60	8 ± 4	100	100	100	—
	15	0	6± 4	95±1	100	—
	30	0	60±11	100	100	—
	45	0	100	100	100	—
	60	19 ± 6	100	100	100	—
House fly (*Musca domestica* L.)	15	0	1±1	85± 8	100	—
	30	0	5±3	100	100	—
	45	3 ± 2	22±7	100	100	—
	60	6 ± 3	63±11	100	100	—
Japanese beetle (*Popillia japonica* Newm.)	15	—	0	75± 8	100	—
	30	—	4±1	100	100	—
	45	—	20±5	100	100	—
	60	—	54±6	100	100	—
Confused flour beetle (*Tribolium confusum* Duv.)	15	—	0	3± 2	100	—
	30	—	0	67±14	100	—
	45	—	0	98± 2	100	—
	60	—	0	100	100	—
American dog tick (*Dermacentor variabilis* (Say))	15	—	0	4± 4	100	—
	30	—	0	59±12	100	—
	45	—	0	94± 4	100	—
	60	—	5±3	100	100	—
Grasshoppers,[b] nymphs and adults	15	—	0	3± 1	78±8	100
	30	—	0	16± 8	100	100
	45	—	0	55± 9	100	100
	60	—	0	88± 4	100	100
Colorado potato beetle (*Leptinotarsa decemlineata* (Say))	15	—	0	0	24±8	100
	30	—	0	41±14	93±5	100
	45	—	1±1	100	100	100
	60	—	13±9	100	100	100

[a] All tests were made with adults except the first half of the results reported for the Mexican bean beetle were obtained with larvae. Six replications of all tests except four for the common malarial mosquito.

[b] 90% *Melanoplus femur-rubrum* (Deg.); 10% *Schistocerca americana* (Drury), *Dissosteira carolina* (L.), *Hippiscus rugosus* Scudd., *Syrbula admirabilis* Uhler., and *Conocephalus strictus* (Scudd.).

100% mortality. With most species complete kill was obtained with longer exposures at temperatures below the point where 100% mortality showed in 15 minutes.

These tests indicate that insects are much less adaptable to extremes of heat than is man. According to Wezler (undated), with humans 50° C. with 50% relative humidity produces symptoms of collapse within about 3 hours, 70° with low humidity can be tolerated for about 1 hour, 130° only about 10 or 15 minutes, and 250° at head level less than 5 minutes.

Investigations were also made to determine the temperature range within a grounded C-47 aircraft fuselage without insulation. On 63 out of 69 test days temperatures within the closed fuselage averaged more than

14° C. higher than outside temperatures. On half the days the temperature reached 49° or higher. Temperature studies were then made at the National Airport, Washington, D. C., in well-insulated, padded Constellation passenger aircraft used by the Military Air Transport Service. Over a 9-day period in August the temperatures inside the closed aircraft ranged from 21° to 36° C. and outside temperatures from 21° to 34°.

These findings along with the information shown in table 1 strongly suggest that many hitchhiking insects of agricultural and public health importance would be killed if exposed within uninsulated aircraft on hot sunny days during the summer months in temperate areas and perhaps throughout the year in the tropics. Quarantine vigilance must not be relaxed, however, because during occasional cloudy or rainy weather or in well-insulated aircraft some insects might survive.

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The Metabolism of Heptachlor by Resistant and Susceptible House Flies[1]

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Abstract

Topically applied heptachlor is rapidly absorbed by heptachlor-resistant house flies and is metabolized to heptachlor epoxide. Identification of the metabolite was made by colorimetric analysis using potassium hydroxide-diethanolamine reagent, and by infrared spectroscopy. The metabolite was found to be as toxic as heptachlor when bioassayed against susceptible house flies. Male flies of the resistant strain are considerably more susceptible to heptachlor than females, yet very little heptachlor was found in the tissues of flies which succumbed to a dosage of 8 μg. of heptachlor per fly.

Susceptible flies also convert heptachlor to the epoxide and the onset of symptoms of poisoning coincides with the appearance of the metabolite. This suggests that death might result from the toxic action of the metabolite and not from the direct effect of heptachlor. Recovery of products resulting from heptachlor metabolism was in no case quantitative and the missing portion has not been characterized. The mechanism of resistance to heptachlor appears to be that of inactivation of the toxic metabolite by solubilization or storage in a non-sensitive tissue such as fat body.

Within the past few years, extensive investigations have been undertaken to determine the physiologic basis of insect resistance to DDT and related compounds. However, little or no information is available on the fate of other chlorinated hydrocarbon insecticides in resistant and susceptible insects.

Using bioassay and colorimetric techniques, preliminary investigations at this laboratory in 1952[3] on the metabolic fate of heptachlor in heptachlor-resistant house flies revealed that a major portion of the absorbed dose was metabolized within 24 hours after application of the chemical. Furthermore, the metabolite appeared to be as toxic as the parent compound when tested against susceptible house flies. This rather unexpected finding led to a detailed study on the metabolism of heptachlor and on the nature of the metabolite.

Isolation and identification of the toxic metabolite was greatly facilitated by knowledge gained from the works of Davidow & Radomski (1953) and Radomski & Davidow (1953) who isolated a metabolite of heptachlor from the adipose tissues of heptachlor-fed dogs and rats, and from a subsequent report by Davidow *et al.* (1953) who detected the same metabolite in the milk of heptachlor-fed cows.

Methods.—The absorption and metabolism of heptachlor by resistant house flies was studied in the following manner: Groups of 50 adult female house flies, 3 to 4 days old, were anesthetized with CO_2 and 8 to 10 micrograms of heptachlor in 1 microliter of benzene was applied to the ventral thoracic region of individual flies. Immediately after application (zero-hour test) and at various intervals thereafter, the flies were first rinsed with three portions of n-hexane to remove any unabsorbed insecticide, then were macerated in a mortar to a fine dry powder with anhydrous sodium sulfate, and were extracted with ether in a Soxhlet apparatus for 8 hours. The extract was evaporated to dryness and the residue was redissolved in 5 ml. of n-hexane (redistilled, commercial grade). This solution was transferred to a chromatographic column containing 15 gm. of adsorption alumina (80–200 mesh) and the column was eluted with additional n-hexane until 100 ml. had been collected. A clean receiving flask was

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[3] Technical Development Branch Summary of Activities No. 32, p. 51; Federal Security Agency, Public Health Service, Communicable Disease Center, Oct.-Dec. 1952.

then placed under the column and the latter was eluted with a mixture of 75% n-hexane and 25% benzene (by volume) until 100 ml. had been collected. Repeated analyses showed that the hexane eluate contained only unchanged heptachlor and the hexane-benzene eluate contained only the metabolite. This simple separation, as outlined by Davidow & Radomski (1953), enabled the study of the rate of heptachlor metabolism in the resistant strain.

Isolation of sufficient quantities of the metabolite for characterization was accomplished by spraying flies, in lots of 100 to 150 each, in a modified space spray chamber originally described by Hoskins & Caldwell (1947). The sprayed flies were held with food and water for 48 hours, after which they were macerated and extracted in a Soxhlet apparatus. Owing to the large amount of impurities in the extracts, it was found necessary to perform some preliminary purification prior to chromatographic separation of heptachlor and the metabolite. For this purpose, the ether extract was evaporated to dryness and the residue redissolved in 50 ml. of n-hexane saturated with acetonitrile. To this were added 150 ml. of acetonitrile saturated with hexane and the mixture was shaken in a separatory funnel for several minutes. After the layers had separated, the lower, acetonitrile phase was collected and the hexane phase was extracted with two additional 100 ml. portions of acetonitrile (Jones & Riddick 1952). The hexane portion successfully removed large amounts of impurities and repeated tests failed to show detectable amounts of either heptachlor or the metabolite in this phase. Hence, this portion was discarded. The combined acetonitrile was then evaporated to dryness following which the residue was redissolved in n-hexane and chromatographed through alumina as outlined above. In this manner approximately 50 mg. of the metabolite was recovered from 40,000 to 50,000 flies.

Colorimetric Procedures.— For the most part, colorimetric analyses of heptachlor and the metabolite were patterned after those described by Davidow (1950), Radomski & Davidow (1953), and Polen & Silverman (1952) with some modifications. Briefly, the tests were carried out in the following manner: For heptachlor analysis, an aliquot of the purified extract was placed in a 10-ml. graduated pyrex test tube and the solvent was evaporated to dryness in a water bath at 40 to 45° C. using a gentle air stream. Care must be taken to avoid excessive air blowing and heating, especially past the point of dryness, in order to minimize losses of heptachlor. One ml. of benzene and 3 ml. of reagent consisting of 1 part diethanolamine and 2 parts of 1N methanolic potassium hydroxide were added and the mixture heated for 30 minutes in an oil bath maintained at 100° C.

The addition of a boiling chip and the directing of a gentle stream of dry air over the mouth of the tube during the first 3 minutes of heating prevents bumping and contribute to smoother evaporation of the solvent. Promptly upon removal from the bath, the mixture was cooled to room temperature by immersing the test tube in tap water, methanol added to bring the volume to 5 ml., and the colored solution read in a Beckman model B spectrophotometer at 565 millimicrons. The purple colored complex was compared with a blank consisting of the reagents alone and similarly treated.

The test for the metabolite was carried out essentially in a similar manner except that the reagent consisted of 1 part diethanolamine and 2 parts of 2N methanolic potassium hydroxide, and the mixture was heated for 30 minutes at 91° C. $\pm$ 1°. The lemon yellow colored complex was read at 415 millimicrons. This peak was chosen in preference to a stronger peak at 285 millimicrons because of lower background interference.

Nature of Reagent.— Polen & Silverman (1952) observed that the color intensity of heptachlor increased with increasing age of the reagent up to 25 to 30 days; after this time, the color intensity remained constant. To overcome this difficulty, these authors suggested heating the freshly prepared reagent for 5 hours at 50° C. to induce a spontaneous aging effect.

This aging phenomenon was confirmed in the present tests but heating the fresh reagent to induce aging was found undesirable as it lasted only 3 to 4 days and gave variable results. Hence, the reagents were aged at least 3 weeks before use.

Calibration Curves.—A plot of absorbancy vs. concentration of heptachlor solutions in benzene revealed noticeable deviations from a Beer's law plot, especially at the region of 0 to 0.125 absorbancy and to a lesser extent at 0.900 and above. No such effect was obtained with the metabolite. These plots are shown in figure 1. For the range of heptachlor concentrations illustrated, greatest accuracy was obtained in the region between 8 and 30 micrograms per ml. of color solution.

RESULTS.—Characterization of the heptachlor metabolite by spectral analysis of the yellow-colored reaction product showed two distinct absorption maxima at 285 and 415 millimicrons, using a Beckman DU spectrophotometer. Identical spectrophotometric curves were obtained with two isomers of a synthesized product chemically identified as heptachlor epoxide and having melting points of 85 to 87° C. and 159 to 160.5° C., respectively (figure 2).

As preliminary data had shown the metabolite highly toxic to susceptible flies, dosage-mortality lines were obtained for the synthesized epoxides and for the metabolite recovered from heptachlor-treated resistant flies. The results of topical application of various dosages of the three epoxides, as well as heptachlor, are shown in figure 3. The proximity of the metabolite's dosage-mortality line to that of the synthesized epoxide, m.p. 159 to 160.5° C., indicates a strong similarity between these two compounds.

A known amount of the fly-epoxide was passed through a 22-bottle counter-current distribution system using n-hexane saturated with 95% methanol as the stationary phase and 95% methanol saturated with n-hexane as the mobile phase (*cf.* Davidow & Radomski, 1953). The concentration of epoxide in each bottle was determined colorimetrically as described previously, and also by bioassay. A graphic plot of the results (figure 4) showed a normal distribution curve between bottles 3 and 15 with maximum concentration in bottle 10. Bottles 1 to 3 contained an oily residue but no epoxide and bottles 15 to 22 were blanks, although slight activity was obtained in the latter in the bioassay tests, due perhaps to the greater sensitivity of the method. Similar determinations made with the synthesized epoxides showed distribution curves

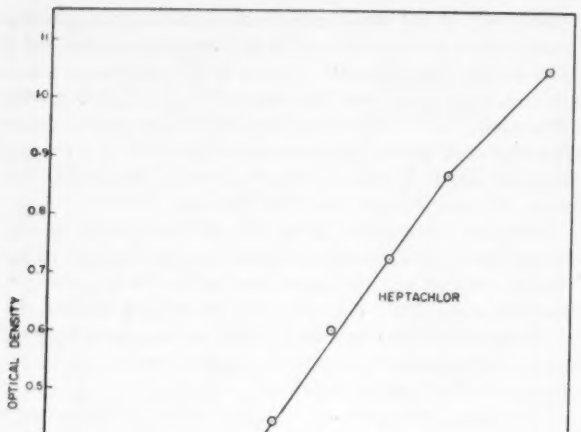

FIG. 1. Calibration curves for heptachlor epoxide (m.p. 159 to 160.5° C.) and heptachlor.

identical with that of the fly extract. However, no distinction could be made in this respect between the low and the high melting epoxide isomers.

Supporting evidence on the identity of the fly epoxide was furnished by infrared spectral analysis. Infrared spectrograms of the heptachlor epoxide isolated from fly tissues were found to be more comparable to those of the synthesized epoxide isomer, m.p. 159° C. (figure 5). In particular, strong similarity was shown by the fly epoxide to the high melting isomer in the 860 to 800 cm^{-1} and 775 to 700 cm^{-1} regions.

Metabolism of Heptachlor in Resistant and Susceptible Flies.—Following topical application of benzene solutions of heptachlor to 3- to 4-day-old adult female resistant flies, absorption and metabolism of the chemical were studied at various intervals ranging from 2 to 72 hours. Temperature and humidity were maintained at 26° C. ±1° and 60 to 65% relative humidity, respectively. The averaged results of eight tests are shown in table 1. It is evident that heptachlor was absorbed fairly rapidly. Thus, 7 hours after application only 6% of the applied dosage was recovered in the external rinse and the chemical was absorbed completely within 24 hours. Small amounts of heptachlor epoxide were recovered at the 2-hour interval and these increased up to 24 hours, after which they remained fairly constant. Repeated applications of 10 micrograms heptachlor per female fly at 24-hour intervals yielded a corresponding increase in heptachlor epoxide in proportion to the cumulative dosage applied. However, the mortality rose from 0% after a single application to 40% after three applications and to 94% when the flies were kept for 2 additional days after the third application. These results are summarized in table 2.

Total recovery of materials was in no case quantitative. Analysis of excreta from heptachlor-treated flies yielded no additional metabolites or unchanged material.

In contrast with the above findings, application of heptachlor epoxide or the fly extract to resistant flies yielded quantitative recoveries of the same compounds. The averaged results of six tests are shown in table 3. It is worth noting that the extracted metabolite was more toxic to the resistant strain than the corresponding synthesized epoxide. This rather curious observation is as yet unexplained and must await further clarification.

The metabolism of heptachlor in susceptible house flies (National Association of Insecticide and Disinfectant Manufacturers' strain) was followed in a manner identical with that described for the resistant strain except for the use of lower dosages. Since the application of dosages in fractions of a microgram entails the use of large numbers of flies, some tests were performed with the space spray technique described earlier. In many tests microcells were used to increase the sensitivity of the method of analysis. Typical results are shown in table 4. Significant amounts of heptachlor epoxide were recovered as early as 2 hours after treatment with heptachlor and these increased with increasing time interval. Here, too, recoveries were not quantitative.

DISCUSSION.—The results obtained in this investigation clearly indicate that house fly resistance to heptachlor does not specifically involve a detoxication mechanism. On the contrary, the conversion of heptachlor to heptachlor epoxide appears to be a process of "autointoxication" since the metabolite (heptachlor epoxide) is as toxic as the parent compound.

Davidow & Radomski (1953) and Radomski & Davdow (1953) have shown that heptachlor epoxide accumu-

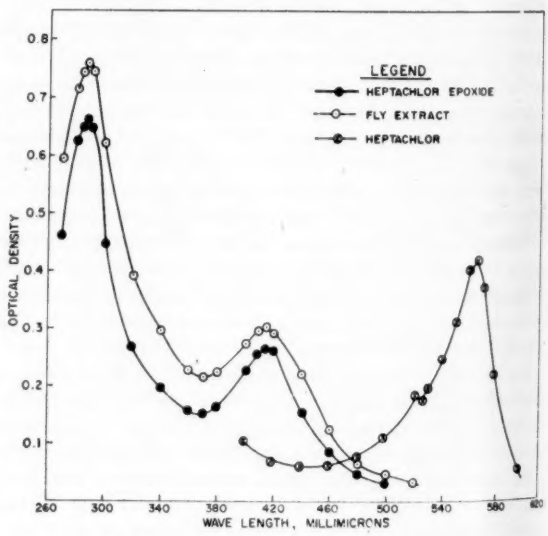

FIG. 2.—Absorption spectra, using a Beckman DU spectrophotometer, of the colored reaction product of heptachlor, heptachlor epoxide, and an extract of heptachlor-treated resistant house flies.

lates primarily in the fat of heptachlor-fed dogs and rats. Considerably lower amounts were found in the liver, kidney, and muscle and none in the brain. Davidow, *et al.*, (1953) also found the same metabolite concentrated in the milk butterfat of a heptachlor-fed dairy cow.

It is well known that many of the chlorinated hydrocarbon insecticides are readily stored in mammalian fat depots. However, little information is available on the role of insect lipids, other than cuticular lipids, in the toxic action of insecticides. Munson (1953), Munson & Gottlieb (1953), and Munson, *et al.*, (1954) studied the lipid content of the American cockroach in relation to the insecticidal action of DDT. These investigations, however, were concerned mainly with the effect of temperature on the holding capacity of lipids for DDT and methoxychlor, and with the effect of preconditioning the test insects at different temperatures on their resistance to various insecticides. Reiser, *et al.*, (1953) found a direct correlation between fat content and survival of boll weevils exposed to toxaphene, dieldrin, EPN, methyl parathion, and calcium arsenate, but the same was true also for untreated controls. No correlation was found between fat content and resistance to toxaphene in seven other insect species collected from cotton. The authors concluded that the higher fat content and increased resistance of late season boll weevils are due to nutritional advantages and are not directly related to insecticide resistance. Mer & Furmaska (1953) also found an increased resistance to knockdown by DDT deposits as a result of addition of 30% butterfat in the diet of *Musca domestica vicina*. The influence of diet on fat content and other pertinent information on insect lipids has been reviewed by Scoggin & Tauber (1950). Recently, Wiesmann (1955) found that the cholesterol content of brain, abdominal nerve cord, and wing muscles of DDT-resistant house flies was two to three times higher than that of susceptible flies. Since cholesterol is known to decrease tissue permeability to DDT, this author concluded that the difference in resistance between the two strains could be attributed, among other things, to a decrease in permeability of nerve tissue. In addition, Wiesmann (1955) reported differences in physical and chemical properties of the lipids between the two strains and a higher total lipid content in the resistance strain. Lower sensitivity to DDT of the thoracic ganglia of resistant flies was previously reported by Pratt & Babers (1953), and the results of Weiant (1955) may be similarly interpreted.

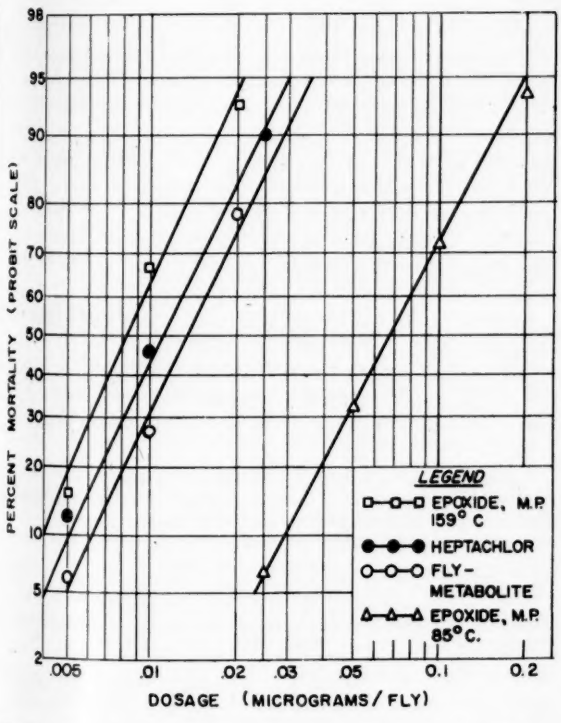

FIG. 3.—Dosage-mortality lines for susceptible house flies (Beltsville strain) resulting from topical applications of heptachlor, two heptachlor epoxide isomers, and an extract of heptachlor-treated resistant house flies.

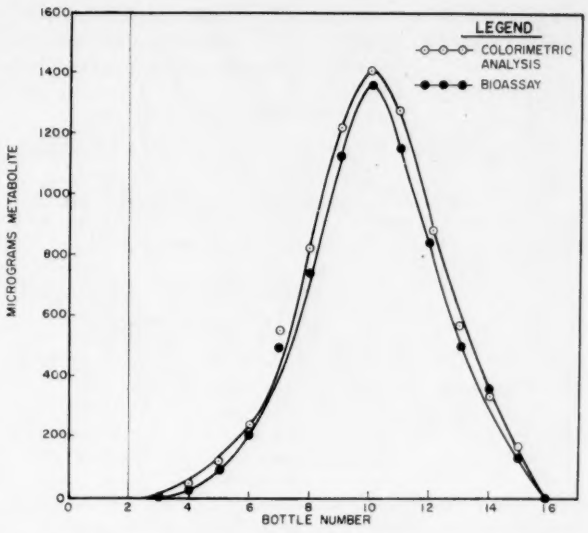

FIG. 4.—Bioassay and colorimetric analysis of an extract obtained from heptachlor-treated resistant house flies and processed through a 22-bottle countercurrent distribution system.

The ability of the heptachlor-resistant strain to withhold large quantities of the toxic metabolite without apparent harmful effect suggests a mechanism of inactivation of the chemical by solubilization or storage in a nonsensitive tissue, *e.g.*, fat body. This hypothesis is somewhat strengthened by the observation that starving heptachlor-treated resistant flies after the epoxide had been formed elicited the onset of strong symptoms of poisoning. Although untreated flies (controls) suffered 20 to 25% mortality after 48 hours of starvation, 70 to 80% of the starved, treated flies succumbed within the same period.

The fact that susceptible house flies also convert heptachlor to the epoxide suggests that death might result from the action of the epoxide and not from the direct action of heptachlor. Numerous observations have shown that there is a latent period between application of heptachlor and the onset of symptoms of poisoning. This phenomenon is not due to slow penetration, since sufficient heptachlor to cause complete mortality of the flies had penetrated as early as 30 minutes after application of 1 micro-

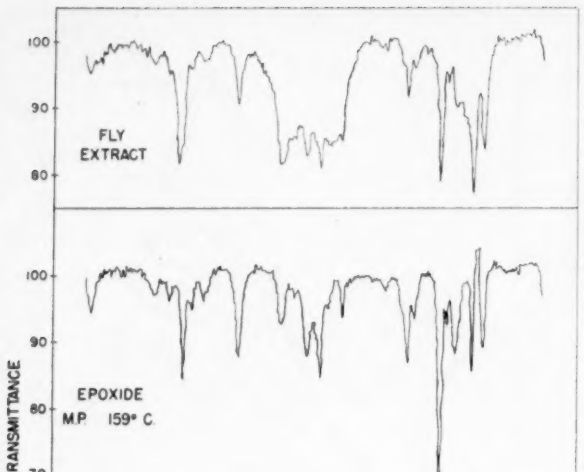

FIG. 5.—Infrared spectra of the house fly extract and the two synthesized heptachlor-epoxide isomers using a Beckman IR-3 spectrophotometer, 0.2 mm. cells, NaCl optics, and approximately 1.5% solutions in carbon disulfide.

gram heptachlor per fly. Symptoms of poisoning begin to appear 1 to 2 hours after application of heptachlor and these symptoms coincide with the appearance of the epoxide in sufficient concentration to cause knockdown and eventual death (table 4). These findings are analogous with those of Harvey & Brown (1951) who observed a latent period of about 2 hours before any rise in O_2 consumption occurred in cockroaches injected with heptachlor.

Table 1.—Absorption and metabolism of heptachlor by resistant house flies at various intervals following topical application of 8.0 micrograms heptachlor per female fly.

Interval to Extraction (Hours)	Recovered (Micrograms/Fly)				Per Cent Recovery[a]
	External Heptachlor	Internal Heptachlor	Internal Epoxide	Total	
0	8.0	—	—	8.0	—
2	2.50	3.68	0.39	6.57	82.1
4	0.95	4.34	0.90	6.19	77.4
7	0.47	3.25	1.50	5.22	65.3
24	0	0.30	3.85	4.15	51.9
48	0	0	4.23	4.23	52.8
72	0	0	4.10	4.10	51.2

[a] Based on 0-hour recovery test.

Table 2.—Metabolism of heptachlor by resistant house flies following repeated applications at 24-hour intervals of 8.5 micrograms heptachlor per female fly.[a]

Cumulative Dosage μg./Fly	Interval to Extraction (Hours)	Recovered (μg./Fly)[b] Internal Heptachlor	Internal Epoxide	Total	Per Cent Recovery	Per Cent Mortality
8.5	24	0.22	4.0	4.22	49.6	0
17.0	48	0.52	7.92	8.54	50.2	12
25.5	72	0.90	11.60	12.50	49.0	40
25.5	120	0.48	12.40	12.88	50.5	94

[a] Controls showed negligible mortalities after 120 hours.
[b] No external heptachlor was recovered.

Table 3.—Quantitative determination of materials recovered and mortalities of heptachlor-resistant house flies following topical application of 7.5 to 8.5 micrograms of the indicated material per female fly.

Material Applied	Interval to Extraction (Hours)	Material Recovered	Amount Recovered μg./Fly	Per Cent Recovery[a]	Per Cent Mortality
Epoxide m.p. 159° C.	0	—	8.50	—	—
	24	Unchanged epoxide	8.40	98.8	2
	48	Unchanged epoxide	7.54	88.7	6
	72	Unchanged epoxide	8.00	94.1	6
Epoxide m.p. 85° C.	0	—	8.30	—	—
	48	Unchanged epoxide	7.65	92.2	2
Fly epoxide[b]	0	—	7.50	—	—
	48	Unchanged metabolite	7.24	96.0	45-60

[a] Based on 0-hour recovery test.
[b] Concentration determined colorimetrically and extrapolated from the epoxide, m.p. 159° C., calibration curve.

Male flies of the resistant strain are not as resistant to heptachlor as female flies. A dosage of 10 micrograms per fly killed 80 to 90% of the males but none of the females in 48 hours. In spite of this high mortality, only 0.2 microgram of heptachlor per male fly was recovered but the epoxide content was 4.0 micrograms per fly. Since 4.0 micrograms epoxide per male fly produces 75 to 85% mortality as determined experimentally, it is apparent that the mortality obtained with the heptachlor-treated flies was due to the epoxide produced and not to heptachlor itself.

Similarly, the high mortality suffered by resistant female flies after three repeated applications of 10 micrograms heptachlor per fly (see table 2) could not have been due to the small amount of heptachlor recovered internally. These findings further indicate that death might re-

Table 4.—Metabolism of heptachlor by susceptible house flies (NAIDM strain) at various intervals following topical application or space spray with heptachlor.

Method of Application	Interval to Extraction (Hours)	Recovered (μg.Fly) External Heptachlor	Internal Heptachlor	Internal Epoxide	Per Cent Recovery[a]	Condition of Flies
Topical	0	1.0	—	—	—	
	2	0.10	0.43	0.20	73	87% knockdown
	4	0	0.48	0.24	72	94% knockdown
	7	0	0.27	0.39	67	95% dead
	24	0	0.14	0.48	62	99 dead
Space Spray	0	0.38	—	—		
	24	0.02	0.16	0.12	79	98% dead

[a] Based on 0-hour recovery test.

sult from the effect of the toxic metabolite and not from that of heptachlor.

The low recoveries obtained following topical applications of heptachlor might be attributable to: (1) volatilization of the compound from the site of application, (2) loss of heptachlor during concentration of the extract, and (3) formation of other metabolites which do not respond to the method of analysis. A check on volatilization of heptachlor was made by applying the chemical to flies previously killed by heat or with chloroform vapors. A loss of 3 to 5% occurred at the 4-hour interval, 10 to 15% after 24 hours and 15 to 20% 48 hours after application. Since all of the heptachlor applied penetrated within 24 hours after application (table 1), loss by volatilization can account for only 10 to 15% of the 10 micrograms applied.

Precautionary measures were taken to minimize losses during concentration of the extracted material. Polen & Silverman (1952) demonstrated that significant losses of heptachlor can occur during evaporation of hexane solutions of the chemical depending on the initial volume of the solution. To obviate such losses they suggested using pentane instead of hexane. In the present work, pentane was found unsatisfactory for the chromatographic separation of heptachlor and the epoxide. In any case, the volume of extracts never exceeded 100 ml. and from Polen & Silverman's (1952) plot it can be seen that loss from such a volume does not exceed 8%. Numerous tests were performed to check on losses during evaporation. It was found that concentrating the extracts on a steam bath at 35 to 40° C. and using a gentle air stream yields almost complete recovery of the chemicals. However, losses up to 25% were encountered if the temperature was raised to 60° C.

This leaves a third possibility, *i.e.*, the formation of other metabolites which do not respond to the method of analysis. From previous experience with other insecticides involving a similar situation, the authors are reluctant at this writing to suggest the formation of unknown metabolites since this assumption would have to be based entirely on analytical differences. Until some sort of characterization of the missing portion can be made it is best to table this question. In this respect, radioactive heptachlor would be extremely useful.

Davidow & Radomski (1953) suggested a mechanism whereby heptachlor is oxidized at the reactive double bond to yield an epoxide; the epoxide is hydrolyzed to a diol, and the diol may split out a molecule of water to yield a phenol. The epoxide was suggested as being a stable intermediate which is retained in the fat because of its high lipoid solubility.

It is conceivable that such a mechanism could occur in the house fly. If the corresponding phenol were formed it could be excreted as a glucoside conjugate or as an ethereal sulfate since Myers & Smith (1953), Smith (1955a, 1955b), and others have shown that many insect species excrete various injected phenols as the corresponding glucoside conjugates and less frequently as the ethereal sulfates. If such were the case, the excretory products of heptachlor metabolism could account for all or part of the missing material. Investigations along this line are contemplated.

ACKNOWLEDGEMENT.—The writers are indebted to the Velsicol Corporation, Chicago, Illinois, for making the infrared analyses and for supplying samples of pure heptachlor and the epoxide isomers.

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Tedion, an Outstanding New Acaricide[1]

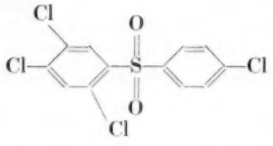
WILLIAM C. FERGUSON[2]

ABSTRACT

Tedion, 2,4,5,4′-tetrachlorodiphenyl sulfone in developmental studies has been outstanding as an acaricide, particularly against *Tetranychid* species of mites. Results of field tests have shown Tedion to be very effective for the control of various kinds of mites on citrus, deciduous fruits and ornamentals. Tedion is usually very slow acting against adult mites; however, its effectiveness is due primarily to its ovicidal action and long residual life against newly hatched larvae. By not being effective against insects, Tedion will not interfere with the mite-insect predator relationship. It has shown no apparent fruit or foliage injury to a wide variety of fruits, vegetables or ornamentals whether treated in the greenhouse or field. Toxicity studies in progress strongly indicated that Tedion has a very low order of toxicity against warm-blooded animals.

Tedion is an acaricide discovered in the laboratories of N. V. Philips-Roxane of the Netherlands for which the U. S. patent rights are controlled by the North American Philips Company. Tedion is apparently a selective acaricide in which tests to date have shown its effectiveness limited primarily to *Tetranychid* mites. Tedion is usually very slow acting against the adult mites present at time of treatment; however, it is unique in its very long residual action against newly hatched larvae and nymphs. Tests to date have indicated that Tedion is ineffective against insects ordinarily used in laboratory studies as well as those of economic importance in the field, including bees. This fact has an important bearing on the ultimate performance of an acaricide in that it minimizes upsetting the natural balance between the mites to be controlled and their predators. To date no plant injury caused directly by Tedion has been reported either in the field or greenhouse. As a matter of added interest, Tedion is currently being sold in France and Holland for the control of mites on a wide range of vegetable crops and fruits grown under glass as well as in the field.

COMPOSITION.—Tedion is 2,4,5,4′-tetrachlorodiphenyl sulfone. The current technical product contains 90% or more of this compound. Its structural formula is represented as follows:



TOXICOLOGY.—All indications to date are that Tedion is quite innocuous to warm-blooded animals. These studies, including a 2-year chronic toxicity program, are still in progress. A summary of the information obtained to date follows:

Acute oral toxicity tests.—1. Rats.—Male albino rats averaging 115 gms. in weight were administered orally dosages of Tedion as high as 14,700 mg./kg. without showing any signs of systemic toxicity or mortalities. 2. Dogs.—Tedion was administered to dogs in dosages as high as 2000 mg./kg. body weight on 4 successive days and were observed for 5 additional days. There were no apparent symptoms of poisoning.

Acute dermal toxicity tests.—Tedion applied to the clipped abdomen of albino rabbits at rates up to 10,000 mg./kg. resulted in only a very mild skin irritation.

Acute eye irritation tests.—A single application of 3 mg. of Tedion instilled into the eyes of albino rabbits resulted in mild irritation. The test animals returned to normal in 24 to 48 hours.

Chronic toxicity studies.—Chronic feeding studies currently underway show no growth depression at 5000 p.p.m. in the diet of rats after 6 months. Another group fed at 20,000 p.p.m. for 8 months resulted in no significant mortality differences from the controls.

Tissue storage.—Rats fed 5000 p.p.m. Tedion in the diet for 1 year had approximately 320 p.p.m. Tedion deposited in the fat. This disappeared after the animals were fed for 30 days on a Tedion-free diet.

The foregoing experiments have been conducted by independent laboratories that specialize in toxicological studies.

BIOLOGICAL PERFORMANCE-LABORATORY OBSERVATIONS.—The biological performance of Tedion in the laboratory has demonstrated a mode of action rather unique among pesticides. With specific reference to the control of *Tetranychus telarius* (L.) it has been demonstrated that adult female mites, although not themselves quickly killed when in direct contact with Tedion, deposited eggs either that did not hatch or were delayed in their normal hatching period. In instances where the eggs had been sprayed with Tedion many were killed while with others the newly emerging larvae after cracking the chorion of the egg died before they crawled free from the egg. Those mites that did completely emerge from the egg were soon killed by the Tedion residue on the leaves.

BIOLOGICAL PERFORMANCE-FIELD OBSERVATIONS.—In the field extensive tests have been conducted for the control of foliage mites on citrus and deciduous fruits. Response to these applications has followed the same pattern already described for the laboratory tests. In the field the residual effectiveness of Tedion has ranged from 30 to over 100 days depending upon the crop and weather conditions. It is important to note that Tedion is effective only against the summer eggs and ineffective against over-wintering eggs; therefore, particularly against European red mite (*Panonychus ulmi* (Koch)) the first application should be made after the overwintering eggs have hatched.

Although data are not presented in this paper, agricultural experiment station workers on the West Coast have reported that Tedion has given excellent control of mites known to be resistant to parathion, ovex and Genite.

RESULTS OF FIELD TESTS.—The field tests reported in this paper were conducted by personnel of the Research Department of the Niagara Chemical Division and, for

[1] Accepted for publication December 12, 1957.

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Table 1.—Citrus red mite control on Valencia oranges, Riverview, Florida.

WP Treatment	Pounds per 100 Gallons	Number of Living Mites per 50 Leaves					
		March 19[a]	March 20	March 29	April 13	April 26	May 16
Tedion 25%	1.0	—	234	42	0	0	3
Aramite 45%	0.66	—	2	0	0	24	210
Untreated	—	460	401	460	312	250	295

[a] Date treatments were applied.

the most part, portray data that are typical of those received from various Federal and State research workers.

In table 1 it is shown that 1 pound of 25% Tedion wettable powder gave a slow kill of the citrus red mite, *Panonychus citri* (McG.) present at the time of treatment but afforded excellent control of the eggs, young larvae and nymphs for a period of at least 54 days. A wettable powder formulation of 45% Aramite gave a quick and complete kill of all active stages of mites within 24 hours after treatment. Aramite showed good residual control of the mites for a period of 38 days following treatment. Although this particular test was abandoned at the end of 58 days, results reported by other workers with citrus red mite indicate consistently that the residual activity of Tedion could be expected to extend well beyond this time.

In the control of two-spotted spider mites, *Tetranychus telarius* (L.), on Better Times roses in the greenhouse, it is shown in table 2 that 1 pound of Tedion wettable powder per 100 gallons of water gave good mite control for a period of approximately 50 days. When the dosage was reduced to one-half pound per 100 gallons, residual life of the Tedion was reduced to 42 days. A wettable powder formulation containing 50% Tedion when used at the rate of one-quarter pound per 100 gallons was not quite so effective as an equivalent amount of the active ingredient applied as a wettable powder formulation containing 25% Tedion. It is believed, however, that the performance of the 50% Tedion wettable powder can be increased by an improved formulation.

For the control of two-spotted spider mites on apples one application of one-half pound 25% Tedion wettable powder applied in the early periods of mite migration up into the trees gave good control for the remainder of the season. One application, under the conditions of this test, gave as good control as two applications at an 8-day interval. In table 3 it is shown that the increase of 25% Tedion wettable powder to 1 pound and used at one or two applications was not significantly better than the one-half pound dosage. The standard treatment of 1.5 pounds 15% Aramite wettable powder also gave good control of this pest.

Table 4 shows the effectiveness of Tedion against the European red mite and the two-spotted spider mite on apples. One pound of 25% Tedion wettable powder per 100 gallons of water applied at the pre-pink period gave good control of European red mites for 82 days during which time the mite population averaged well under one mite per leaf. During this period the two-spotted spider mites were controlled satisfactorily. In comparison a one-half pound dosage was nearly as effective as the 1 pound rate. Two applications of one-half pound 25% Tedion wettable powder applied at the pre-pink and 10-day periods gave excellent control of both species of mites throughout the season. An additional application of Tedion at the second cover period gave no increased benefit.

PHYTOTOXICITY.—Tedion has been exceptionally safe on plants whether used in the greenhouse or in the field under a wide range of geographical conditions. To date there has not been a single report of any visible injury to any plant on which it has been used.

Table 2.—Two-spotted spider mite control on Better Times roses. Middleport, New York.

WP Treatment	Pounds per 100 Gallons	No. of Mites per Leaf on Days After Treatment Indicated											
		1	4	9	19	28	37	39	42	44	46	49	51
Tedion 25%	1.0	2.4	2.8	0.7	0.1	1.1	0.5	0	0.1	5.3	2.6	3.4	6.0
Tedion 25%	0.5	6.6	1.9	1.6	1.5	1.3	2.6	5.3	3.5	14.1	14.4	25.9	19.6
Tedion 50%	0.25	0.2	5.1	1.4	4.0	5.5	2.3	16.7	10.3	19.4	13.8	9.4	23.1
Untreated		12.4	19.5	30.1	16.6	14.0	15.7	13.9	8.9	32.5	46.8	81.4	54.3

Table 3.—Two-spotted spider mite control on McIntosh apples. Niagara Research Farm, Gasport, N. Y.

WP Treatment	Pounds per 100 Gallons	July Dates Applied	Number Mites per Leaf on					
			July 19	July 25	Aug. 6	Aug. 20	Aug. 30	Sept. 10
Tedion 25%	0.5	15	6.0	0.1	0	0	0	0.5
	0.5	15						
	0.5	23	—	1.1	0.5	0.03	0	0.03
	1.0	15	2.5	0.6	0.1	0	0	0
	1.0	15						
	1.0	23	—	0.4	0	0	0	0
Aramite 15%	1.5	15	1.6	0.4	2.5	1.6	1.8	2.2
	1.5	15						
	1.5	23	—	0.03	1.6	0.1	1.1	0.2
Untreated	—	—	3.1	10.9	42.2	11.1	9.4	3.1

Table 4.—Mite control with 25% Tedion wettable powder on Baldwin apples, Niagara Research Farm, Gasport, N. Y.

Pounds per 100 Gal.	Period of Treatment[a]	Number of Mites per 100 Leaves										
		European Red Mite							Two-Spotted Spider Mite			
		6/20	6/28	7/7	7/17	7/24	8/6	8/13	7/17	7/24	8/6	8/13
1.0	Pp	17	5	0	57	10	100	27	20	10	50	17
0.5	Pp	0	4	0	300	40	270	150	130	0	63	17
0.5	Pp+10D	4	0	0	0	0	10	10	0	0	0	0
0.5	Pp+10D+2C	—	—	—	0	7	30	17	0	0	27	0
None	—	47	24	80	1650	690	2080	270	450	106	290	17

[a] Pp = pre-pink; 10D = 10-day spray; 2C = second cover spray.

RESIDUE ANALYSIS.—A satisfactory method of analysis for Tedion residues on fruit and foliage has been developed. An extensive residue program is underway, the results of which will be available at a later date.

CONCLUSIONS.—Results of field tests have shown Tedion effective as an ovicide-larvicide in the control of various mite species on citrus, deciduous fruits and ornamentals. Although not specifically shown in this report, Tedion has given excellent control of mites known to be resistant to parathion, ovex and Genite. Tedion has caused no apparent injury to a wide range of fruit and vegetable crops grown in the field as well as a wide variety of ornamentals grown in the greenhouse. Tedion has shown an extremely low-order of toxicity against warm-blooded animals.

Ethion, A Promising New Acaricide and Insecticide[1]

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ABSTRACT

Ethion is identified chemically as O,O,O′,O′-tetraethyl S,S′-methylene diphosphorodithioate and has been known under the name of Niagara 1240. Results from 2 years of field tests demonstrated that the compound was effective against two species of aphids on apples, two species of foliage mites and the codling moth. On apples 2 pounds of 25% wettable powder per 100 gallons, applied when trees were either in dormant or delayed-dormant stage of development gave good control of the apple grain aphid, *Rhopalosiphum fitchii* (Sand.) and the rosy apple aphid, *Anuraphis roseus* Baker. One pound of ethion wettable powder per 100 gallons applied in pre-pink or in the early post-bloom period provided control of the European red mite, *Panonychus ulmi* (Koch). When applied in mid-season ethion at the rate of 1 pound of 25% wettable powder was also effective for the control of the two-spotted spider mite, *Tetranychus telarius* (L.).

The use of ethion at the 2-pound rate in all post-bloom sprays resulted in control of the codling moth, *Carpocapsa pomonella* (L.), equal to such standard control chemicals as lead arsenate, DDT and parathion. Lower concentrations were not commercially effective.

Ethion (also designated at various times as Niagara 1240 and Nialate) identified chemically as O,O,O′,O′-tetraethyl S,S′-methylene diphosphorodithioate is an organic phosphate discovered and developed in the Research Laboratories of the Niagara Chemical Division at Middleport, N. Y. Like many of the pesticides in this chemical area, ethion may be classed as a cholinesterase inhibitor. The acute oral toxicity to rats (LD_{50}) of material representative of current production has been found to be 179 mg./kg. Feeding tests that must be conducted with such materials before tolerances can be established are in progress and results should become available during the 1958 season.

Ethion has now been evaluated in the field over a 2-year period. Those investigating the activity of the material have included state and federal investigators, as well as several members of the Research Department of the Niagara Chemical Division.

Ethion has been tested against most of the important pests associated with deciduous fruits, citrus, cotton, forage and against some of the soil insects. It has demonstrated excellent activity against most of the tetranychid foliage mites, some species of aphids on deciduous fruits and against the codling moth, *Carpocapsa pomonella* (L.). This paper will be devoted particularly to experiments conducted at the Niagara Research Farm, Middleport, N. Y., for the control of insects on deciduous fruits.

METHODS AND MATERIALS.—All compounds were applied with a single nozzle spray gun operated at about 500 p.s.i. pressure. Ten gallons of diluted spray per tree were applied in the prebloom treatments and this was increased to about 15 gallons for post-bloom applications. Chemicals employed have been designated in the tables as wettable powders (WP) and emulsifiable concentrates (E). The numbers associated with the formulation designations refer to the percentage composition of active ingredient in the case of wettable powders and to the number of pounds of active ingredient per gallon in the case of the emulsifiable concentrates.

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Table 1.—Results of experiments to control aphids on apples. Middleport, N. Y.

Material and Pounds or Quarts per 100 Gal.	Time of Treatment		Apple Grain Aphid/100 Buds		Rosy Apple Infestations per Tree Plot B June 26
	Plot A[a] April 16	Plot B[b] May 8	Plot A May 4	Plot B May 9	
			1956		
Ethion 25 WP 0.5	Dormant	Del.Dor.	23	33	19
1.0	Dormant	Del.Dor.	7	31	19
2.0	Dormant	Del.Dor.	1	7	10
DN-40 WP 3.0	Dormant	—	2	—	—
1.5	Dormant	—	2	—	—
BHC 10 WP 2.0	—	Del.Dor.	—	11	19
Untreated			166	107	165
			1957		
	April 15		April 24		Per Cent Blossom Clusters Infested May 8
Ethion 25 WP 2.0	Dormant	—	0.3	—	1
2 E 1	Dormant	—	0	—	1
DNBP 2	Dormant	—	0.2	—	1
Untreated			89.1	—	47

[a] Variety McIntosh.
[b] Variety Cortland.

The active ingredient in the DN-40 formulation was dinitro ortho cresol while in DNBP it was the triethanolamine salt of 4, 6-dinitro-2-*sec*-butylphenol. Aramite is identified as 2-(p-tert-butylphenoxy) isopropyl-2-chloroethyl sulfite. The miscible oil used in delayed-dormant treatments contained 98% of petroleum oil.

For the several experiments conducted it was necessary to make treatments at quite different times during the growing season. These periods have been referred to in the tables as "Dor." (Dormant), "Del.Dor." (Delayed Dormant), "Pp" (Pre-pink), "10D" (10-Day or curculio spray), and "2C" as (Second Cover Spray).

The general experimental plan for tests involving aphids or mites was to utilize single tree plots and replicate them from three to five times. Treatments directed to the control of codling moth where infestations were light were replicated three times with each replicate comprising from 23 to 30 trees and including the varieties Baldwin, Cortland and Wealthy. For situations involving much heavier populations the number of trees per replicate was reduced to three to five.

Aphid counts were made in several ways. In 1956 the apple grain aphid, *Rhopalosiphum fitchii* (Sand.), on any given treatment was determined by counting the actual number of aphids on 100 buds, chosen at random from several trees. Although limited sampling revealed that the total aphid population was made up of about 90% apple grain aphids and 10% rosy apple aphids, *Anuraphis roseus* Baker, total count was recorded in terms of the former. In 1957 under conditions of apparent lighter infestation, 1000 rather than 100 buds were counted.

In 1956, rosy apple aphid control was recorded in late June even though treatments were made in the delayed-dormant period. The extent of these infestations was determined by counting the number of "aphid clusters" per tree that could be readily observed when walking around the outside of the tree. In 1957 the per cent of rosy apple aphid infestation was arrived at by sampling 100 blossom clusters and determining the number on which aphids were present.

All mite counts were made at intervals of from 1 to 2 weeks for the duration of the test. It was customary to sample 10 leaves per tree and usually a total of 30 leaves per treatment. Early in the season primary leaves close to fruit spurs were chosen but when terminal growth was completed it was customary to select about the 10th leaf back from the end of any new growth. No effort was made to differentiate between nymphs and adult mites. Counts were made only of crawlers on the underside of the leaf unless otherwise noted.

All data relative to the control of codling moth were obtained at harvest. In 1956 fruit on all trees was picked, then a composite sample comprising from 400 to 500 apples for each treatment was scored as to successful entry by codling moth larvae. No effort was made to

Table 2.—Results of tests to control the European red mite on Baldwin apple. Middleport, N. Y.

Material and Pounds or Pints per 100 Gal.	Period of Treatment	Mites per Leaf on						
		6/20	6/28	7/7	7/17	7/24	8/6	8/13
					1956			
Ethion 25 WP 0.5	Pp	0	0	0	0.2	0.3	0.8	2.2
1.0	Pp	tr.[a]	tr.	tr.	0.1	0.2	0.3	0.1
2.0	Pp	0	0	0	0	0.1	0.1	0.1
0.5	Pp+10D	0	tr.	0	0.8	0.2	0.2	1.4
0.5	Pp+10D+2C	—	—	—	0.3	0.7	1.7	0.9
Untreated		0.5	0.2	0.8	16.5	6.9	20.8	2.7
		6/11	6/24	7/1	7/10	7/16	7/23	7/31	8/12
					1957				
Ethion 25 WP 0.5	Pp	0	tr.	0.2	1.9	2.8	20	5.4	1.8
1.0	Pp	0	0.1	0.0	2.1	3.3	6.3	1.9	1.0
0.5	Pp+10D	0	0	0.2	2.2	2.9	10.9	0.9	0.5
0.5	Pp+10D+2C	0	0.5	tr.	0.4	2.0	1.5	0.9	1.1
1.0	Pp+10D	0	0.2	0	0.5	1.4	4.6	0.5	1.7
1.0	2C	2.1	1.9	0.3	2.9	0.2	1.3	0.5	0.4
Ethion E 4 0.5	Pp	0	0.1	0.1	1.6	0.5	11.2	1.1	1.2
Ovex 50 WP 0.5	Pp+10D+2C	0	1.0	0	0	0.6	0.4	0.2	1.2
Miscible oil 24.0	Del.Dor.	0	0.1	0.1	0.2	1.0	1.0	0.3	0.8
Untreated		10.5	1.7	1.4	6.1	7.4	10.5	3.3	1.7

[a] Less than 0.05 mites per leaf.

Table 3.—Results of tests to control the two-spotted spider mite on McIntosh apples. Middleport, N. Y.

Material and Pounds or Pints per 100 Gal.	Application Date	Mites per Leaf					
		8/17	8/21	8/27	9/4	9/11	9/17
				1956			
Ethion 25 WP 0.5	8/15	—	1.2	0.6	0.3	10.7	1.1
1.0	8/15	—	0.3	0.1	tr.	0.8	0.8
Ethion 2 E 1	8/15	—	0.4	0.1	0.1	1.9	1.3
Aramite 15 WP 1.5	8/15	1.2	1.2	0.2	0.7	13.8	0.7
Untreated	—	—	—	4.3	5.4	36.8	22.5
		7/19	7/25	8/6	8/20	8/30	9/10
				1957			
Ethion 25 WP 0.5	7/15	0.2	0.1	0.9	1.5	0.8	1.3
0.5	7/15, 7/23	—	0.5	0	1.0	0.6	tr.
Ethion E 4 0.25	7/15	tr.	0.2	7.5	4.6	20.6	11.3
0.25	7/15, 7/23	—	tr.	tr.	0.2	0.3	0.2
Ethion 25 WP 1.0	7/15	tr.	0.1	1.3	1.0	6.6	0.4
1.0	7/15, 7/23	—	0	0	0	0	0.4
Ethion E 4 0.5	7/15	0.2	0.1	0.1	3.4	0.2	2.2
0.5	7/15, 7/23	—	tr.	tr.	0	0	0
Aramite 15 WP 1.5	7/15	1.6	0.4	2.5	1.6	1.8	2.2
1.5	7/15, 7/23	—	tr.	1.6	0.1	1.1[a]	0.2
Untreated	—	3.1	10.9	42.2	11.1	9.4	3.1

[a] All mites found on one of the 30 leaves sampled.

record more than one entry per apple. In 1957 the trees were sampled by picking a minimum of a bushel of fruit per tree, taking care to pick at all heights. The latter technique resulted in a several-fold increase in the number of apples scored for each treatment. Also it should be noted that in those experiments where the infestation of untreated fruit was less than 20% that "Successful entry" and "Attempted entry" were grouped under the one category of "per cent injured."

RESULTS AND DISCUSSION.—Results of early season experiments for the control of aphids are summarized in table 1. Dormant treatments of ethion at the 2-pound per 100 gallon rate resulted in control of the apple grain aphid equivalent to that obtained by 1.5 pounds of DN-40 or 2 quarts of the DNPB (39% active ingredient) formulation. The same ethion treatment made at the delayed-dormant period was fully as, or possibly slightly more, effective than 2 pounds of BHC, of 10% gamma isomer content. For the control of rosy apple aphid, studies in 1956 showed that delayed-dormant treatments of ethion at the 2-pound rate were as effective as the BHC standard when measured in terms of population buildup in late June. In 1957, under conditions of a rather light infestation, an application of ethion in the dormant period, equalled the DNBP standard. In the latter case the per cent of blossom clusters having aphids was used as the criterion for comparision. Considering the results obtained in all aphid experiments, it appears that it would not be wise to use less than 2 pounds of 25% ethion wettable powder or its equivalent in active ingredient as an emulsifiable concentrate.

Results of 2 year's testing of ethion for the control of the European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.), are summarized in tables 2 and 3. In 1956 a single application of as little as 0.5 pound of ethion wettable powder in the pre-pink (overwintering eggs had started to hatch) afforded good control of the European red mite through July. Increasing the rate to 1 or 2 pounds per 100 gallons resulted in even longer residual control. It appeared that if a grower chose to make a single pre-bloom application it would be wise to employ the 1-pound rate. If aphids had been a problem and the winter egg population of mites had been heavy then the 2-pound rate would be worthy of consideration. The use of one pre-bloom treatment plus either one or two post-bloom applications did not offer the pronounced advantage in control that might be expected.

In 1957 a single pre-bloom application of the half-pound rate provided good control for a few weeks but possibly it was not as residual as in 1956. The addition of one post-bloom spray helped very little but two post-bloom sprays did provide an effective program for the season. Again, increasing the rate of ethion in the pre-bloom spray to 1 pound resulted in better residual activity. A single post-bloom spray (second cover) at the 1-pound rate and under conditions of low populations reduced the mites to a safe level but there is some question as to the advisability of withholding treatments until an

Table 4.—Results of tests to control the codling moth on apples. Middleport, N. Y.

Material and Pounds per 100 Gallons[a]	Codling Moth—Per Cent Successful Entry		
	McIntosh		Golden Delicious
	1956	1957	1957
Ethion 25 WP 0.5	51.0	—	—
1.0	7.4	10.6	—
2.0	1.7	2.2	2.6
DDT 50 WP 2.0	1.1	—	—
Lead arsenate 3.0 + lime 3.0	—	1.1	—
Untreated	60.0	42.6	59.6

[a] In 1956, seven applications were made on the following dates: 6/7, 6/15, 6/29, 7/13, 7/26, 8/14, 8/29; in 1957 eight applications were made as follows: 5/22 (calyx), 5/29, 6/10, 6/20, 7/11, 7/23, 8/6, 8/20.

Table 5.—Results of tests to control the Codling moth on apples, Middleport, N. Y.

Material and Pounds per 100 Gal.[a]		Codling Moth Control on: Baldwin No. Examined	Baldwin Per Cent Injured	Cortland No. Examined	Cortland Per Cent Injured	Wealthy No. Examined	Wealthy Per Cent Injured
Ethion 25 WP	1	1048	2.1	1012	3.4	2273	1.2[b]
	2	1317	0.3	1063	1.4	2376	0.3[b]
Parathion 15 WP	2	1169	0.9	1032	0.3	1232	0.8
Lead Arsenate+Lime	3+3	1204	1.5	1115	0.9	1510	1.0
DDT 50 WP	2	1576	0.7	1181	0.3	1463	0.7
Untreated		941	10.5	1187	12.6	2574	16.7

[a] Nine applications made on the following dates: 5/21 (calyx), 6/3, 6/11, 6/24, 7/5, 7/17, 7/25, 8/7, 8/21.
[b] Phytotoxicity observed.

appreciable infestation has developed. The miscible formulation appeared to be equal to the wettable powder in effectiveness when compared on the basis of equal active ingredient.

In 1956 experiments for the control of the two-spotted spider mite were limited to single treatments to established infestations. The half-pound rate of ethion wettable powder provided marginal initial control and although applied in mid-August it was not sufficiently residual to keep populations at a low level for the remainder of the season. Increasing the rate to 1 pound resulted in better initial activity and the treatment remained effective for the remainder of the season. In 1957 all ethion treatments, with the possible exception of the low concentration of the miscible formulation, were effective initially and residually. The use of two treatments at intervals of about 1 week resulted in nearly complete control. This approach is worthy of further study.

Summaries of the data from tests utilizing ethion for the control of codling moth are given in tables 4 and 5. Results in table 4 were accumulated over a 2-year period in an orchard that previously had been neglected and in which codling moth had developed in appreciable numbers. Results of a dosage study demonstrated rather conclusively that 25% ethion wettable powder at the half-pound rate (per 100 gallons) was virtually ineffective. The 1-pound rate displayed marginal effectiveness but the 2-pound rate was about as effective as the DDT or the lead arsenate-lime standards. Results of tests under conditions of a much lighter infestation are given in table 5. Again it is evident the 2 pounds of 25% ethion wettable powder performed about as well as the DDT, parathion or lead arsenate-lime standards. It is to be noted that ethion did have phytotoxic effects on the variety Wealthy but that it was quite innocuous to McIntosh, Golden Delicious, Baldwin and Cortland.

While data have not been presented, rather extensive tests were conducted with ethion for the control of the apple aphid, *Aphis pomi* DeG.; plum curculio, *Conotrachelus nenuphar* (Hbst.) and the red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.). The compound did not provide commercial control of these species.

Serpentine Leaf Miner: Brief History and Summary of a Decade of Control Measures in South Florida[1]

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ABSTRACT

Insignificant infestations of the serpentine leaf miner, *Liriomyza pusilla* (Meig.), previous to 1945 increased to destructive populations in 1947 and in later years. Infestations occur on beans, cabbage, okra, peas, potatoes, tomatoes and other vegetable crop plants. Many pre- and past-DDT developed toxicants were used in field experiments to determine their effectiveness. Certain of the chlorinated hydrocarbon insecticides such as chlordane, lindane, toxaphene and aldrin, were effective in initial experiments but become ineffective after 1 to 3 seasons. Certain of the phosphorus insecticides such as parathion, EPN and Diazinon, gave outstanding to excellent prevention of leaf mines. There is some evidence that parathion is not so effective after 10 years as it was initially. Diazinon is currently recommended.

Infestations of the serpentine leaf miner, *Liriomyza pusilla* (Meig.), which had been low for years, increased to a high level in 1945 and caused severe destruction in 1947. Beans, cabbage, okra, peas, potatoes, squash, tomatoes, turnips and other plants were injured. Large fields of potatoes were injured so severely that the plants became brown as if burned and individual plants frequently perished.

Field plot experiments with various insecticides in 1946 and since that time have yielded considerable data on prevention of the mines caused by this insect. Some of the insecticides were occasionally effective in initial tests and were recommended to growers, but were subsequently superseded by more effective toxicants which in turn became ineffective and were superseded by others.

METHODS.—All discussions herein refer to potatoes and tomatoes in field-plot experiments. Insecticides were

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[2] Acknowledgment is made of the work by R. M. Baranowski in the 1956–57 season.

added to the recommended fungicides and were applied in spray form by power machines similar to those employed commercially. High gallonages (75 to 185) averaging about 125 for potatoes and 165 per acre for tomatoes were applied to cover and to protect the plants from diseases, primarily late blight.

All figures given below as to the amount of insecticide refer to pounds or ounces of the formulation in 100 gallons of the spray mixture. All check treatments were fungicidal applications without the addition of an insecticide. Insecticides were applied in the amounts ordinarily recommended, except as indicated. Insecticidal applications were made at weekly intervals during periods when the insect was present.

Data were taken on two or more occasions for each experiment. Mines were usually counted on the 4th or 5th leaf from the top of the plants. Four leaves per plot were the usual number sampled.

Experimental plots of potatoes were grown at one farm and of tomatoes on another farm about 10 miles distant (except in 1947–48 when they were on the same farm).

Although the numbers of mines were counted, results are expressed as percentages of control (table 1) as computed by the Abbott (1925) formula.

RESULTS.—The earliest data from an experiment were taken in 1946 and involved fungicides listed by Ruehle (1946) and showed that the number of mines were controlled to some degree by certain metallic salts, coppers and a zinc chromate material. More mines were found on plants sprayed with the carbamates than with other materials. Plants sprayed with DDT had slightly fewer mines than those not sprayed with DDT.

In 1946–47 BHC and chlordane were first tested. Chlordane gave very satisfactory control and was recommended for control of mines, Wolfenbarger (1947). Lead arsenate combined with nicotine suppressed considerable development of leaf mines.

In 1947–48 lindane, parathion and toxaphene were used in experiments. Parathion (25%) wettable powder at 2 pounds was outstanding in control of mines in a severe infestation. Lindane and toxaphene also gave very favorable results. DDT alone and in combination with nicotine sulphate, lead arsenate, TEPP, calcium arsenate combined with nicotine sulphate, methoxychlor and TDE were only partially effective.

In 1948–49 parathion, toxaphene and aldrin were outstanding in preventing leaf mines. Chlordane gave fair control. DDT and calcium arsenate were slightly effective in preventing leaf mines. Toxaphene and parathion, therefore, were recommended for control of the mines.

In 1949–50 parathion was again the most outstanding insecticide in the control of mines. Dieldrin, aldrin, toxaphene, and chlordane combined with TEPP were also effective. TEDP and Metacide gave fair control. EPN, tested for the first time this season, gave satisfactory control of leaf mines. Bulan and calcium arsenate were ineffective in the prevention of leaf mines.

In 1950–51 leaf mines were not abundant on potatoes, owing possibly to the use of so much parathion in the area by growers. Data from the experiment on tomatoes showed that EPN, Metacide, and demeton gave high degrees of control. Aldrin and dieldrin controlled about one-half and malathion about one-third of the mines as compared with the check.

In 1951–52 EPN gave considerable control of leaf mines, although Metacide and demeton gave high degrees of control (parathion was not used in the experiments this season). Isodrin, Dilan and two formulations of malathion gave only moderate degrees of control.

In 1952–53 the new toxicants tested were methyl demeton, schradan, heptachlor, NPD, Perthane and Strobane. These materials were not effective. Parathion and EPN again were the most effective, with parathion the more effective of the two. Chlordane, Dilan, dieldrin and endrin were applied in amounts of about $\frac{1}{3}$ to $\frac{1}{2}$ more than is ordinarily used. The results, however, were unsatisfactory.

In 1953–54 a series of parathion dosages was tested consisting of 1, 2, and 4 ounces of technical material in wettable powder and liquid (water base) formulations. Each successive higher dosage prevented leaf mines more effectively than the succeeding lower one and the wettable powder was slightly more effective at each concentration than the liquid material. EPN (27%) wettable powder with one-half pound of DDT gave 97% control. Combinations of 15% parathion, 12 ounces in wettable powder form, with DDT, endrin, TDE or Perthane at one-half the usual dosage were more effective than 1 pound of parathion, alternating with full amounts of the insecticides usually applied. Calcium and lead arsenates gave control of about 50% of the mines.

In 1954–55 two formulations of water base material and an emulsion concentrate of parathion were tested and found to be practically equally effective. Thimet, Chlorthion and Diazinon were used in initial tests and gave 92, 42 and 0% control, respectively. Lack of control by the sample of Diazinon was due to defective formulation, according to the manufacturer. Parathion (15%) wettable powder, 12 ounces, with one-half the usual dosages of endrin, toxaphene, DDT and TDE gave 96, 93, 54 and 63% control, respectively. EPN (27%) wettable powder at 8 ounces in each of two treatments, gave 23 and 37% control, respectively.

In 1954–55 a water base formulation of parathion compared about equally with wettable powder in a series of dosages. Diazinon emulsion concentrate was the most effective treatment in control of leaf mines. Of the three new materials tested, Dipterex was fairly effective and Dow ET-57 (sampled as Dow ET 14), and Phosdrin were ineffective. Lead arsenate at 4, 16 or 64 ounces combined with 15% parathion wettable powder, 12 ounces, gave no practical increase in effectiveness. EPN (17%) wettable powder, at 1 pound gave 77% control.

In 1956–57 two new insecticides, Thiodan and ethion

Table 1.—Per cent control of the serpentine leaf miner with parathion during the period, 1947–57.

CROP	SEASONS OF								
	47–48	48–49	49–50	50–51	52–53	53–54	54–55	55–56	56–57
Potato	93[a]	99	95	95	95	—	—	—	73[a]
Tomato	81[a]	94	98	—[b]	90	90	86	93	65

[a] Two pounds (25% material, WP, in 1947–48; 15% material, WP, in 1956–57), 1 pound for all other data reported here.
[b] Not used; not used on either crop in 1951–52.

(methanedithiol, bis (O,O, diethyl phosphorodithioate) were tested. Ethion combined with zineb on potatoes was as effective as parathion and will be used in further experiments. Guthion and Thimet were effective in control of the leaf miner on potatoes. Diazinon can be recommended for control of the leaf miner following its approval by Federal regulatory agencies.

A summary of the control of leaf miners by parathion over the period 1947–57 is given in table 1.

DISCUSSION.—Initial experiments with chlordane, BHC toxaphene, lindane, parathion, demeton, aldrin, Metacide, dieldrin, endrin, Diazinon, Guthion and Thimet gave outstanding to promising suppression of mines of the serpentine leaf miner. Initial or repeated tests with DDT, TEPP, TDE, methoxychlor, Bulan, malathion, Isodrin, methyl demeton, schradan, heptachlor, Perthane, Strobane, Dilan, endrin, Chlorthion, Dipterex, Dow ET-57, Phosdrin and Thiodan showed that they were ineffective or gave little promise of controlling the serpentine leaf miner. Effectiveness of chlordane, BHC, aldrin and toxaphene appeared to have been lost after the first or second season. Demeton, Metacide and EPN also appeared to become ineffective. Lowered percentages of control by parathion in the season 1956–57 given above and adverse reports by growers may indicate that parathion is no longer as effective as formerly. Certain combinations of toxicants, such as parathion and toxaphene or parathion and endrin, have indicated additive effects in preventing leaf mines.

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Tests with Bayer 21/199 for the Control of Cattle Grubs[1]

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ABSTRACT

South Dakota cattle that had been exposed to infestation by *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.) were sprayed with Bayer 21/199 in three concentrations—0.25%, 0.5%, and 0.75%. Of 48 sprayed animals 46 were grub-free, whereas the untreated control animals averaged 44 grubs each. Oral doses of 25 mg./kg. were less effective than sprays, but resulted in partial control. These tests demonstrated that first-instar larvae of both species of *Hypoderma* can be controlled systemically by concentrations of the spray that do not apparently harm the cattle.

The effectiveness of Bayer 21/199 as a systemic insecticide when applied as a spray for the control of the common cattle grub (*Hypoderma lineatum* (De Vill.) has been reported by Brundrett *et al.* (1957). It was shown by those tests that, under Texas conditions, the first-instar larvae were almost completely prevented from appearing in the backs by spraying the cattle with 0.75% Bayer 21/199 thirty days before the larvae were due to reach the backs. The insecticide was presumably absorbed by the skin and translocated to the tissues in which the grubs were located.

As soon as the preliminary data from Brundrett's test became available, other tests were planned with larger numbers of cattle and other concentrations of the insecticide. Yearling Hereford cattle were shipped to Kerrville from Highmore, South Dakota, since cattle from this region were known to be heavily and rather uniformly infested with both *Hypoderma lineatum* and *H. bovis*.(L.)

On November 19, 1956, a total of 48 animals were sprayed with three concentrations of 21/199—0.25%, 0.5%, and 0.75%—and 16 untreated animals were designated as controls. At each concentration half the cattle were sprayed with an emulsion and the other half with a suspension. Like all range cattle from northern States, these animals had a very thick, water-repellent coat of winter hair. It was necessary to apply excessive quantities of the sprays at high pressure in order to penetrate the hair and wet the skin. A power sprayer was operated at a pressure of 300 pounds per square inch, and a two-nozzle spray gun equipped with No. 3 discs was held as close to the animals as they would permit. About 1½ gallons of spray material was applied to each animal. The runoff from such heavy applications was great, but examination indicated thorough wetting of the skin. It was noted that the emulsions reached the skin better than the suspensions.

On December 18 another group of eight animals was given 21/199 orally. Four received 25 mg./kg. as a single dose and the other four animals received 5 mg./kg. daily for 5 days.

All the cattle were held in a feed lot and provided a ration that gave an average weight gain of 1 to 1½ pounds per day. Encysting grubs were first noted in the backs the first week of January, and all grubs were counted on January 18, 1957. These counts were repeated at intervals of 2 to 3 weeks until April 26, when it was obvious that no new grubs were appearing in the back. At the time of the last count the remaining grubs were expressed, and the cattle were moved from the feed lot to pasture. Examinations during May and June confirmed the belief that no new grubs appeared in either treated or untreated cattle after the count on April 26.

The location of each grub was recorded on a chart to provide a positive record of its appearance and disappearance. In this way a cumulative count of the total number of grubs in each animal was obtained on each date. For the treated animals this record was highly accurate, but it was slightly less so for the untreated ani-

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Table 1.—Cattle grubs in the backs of South Dakota yearling cattle treated with Bayer 21/199.

Treatment	Number of Animals		Cumulative Number of Grubs per Animal						
	In test	Infested	Jan. 18	Feb. 5	Feb. 20	Mar. 6	Mar. 19	Apr. 4	Apr. 26
Sprays									
0.75% Suspension	8	0	0	0	0	0	0	0	0
Emulsion	8	0	0	0	0	0	0	0	0
0.50% Suspension	8	2	0	0.4	1	1	1	1	1
Emulsion	8	0	0	0	0	0	0	0	0
0.25% Suspension	8	0	0	0	0	0	0	0	0
Emulsion	8	0	0	0	0	0	0	0	0
Oral doses									
25 mg./kg.	4	2	0	1.5	2.2	3.5	4.0	4.0	4.0
5 mg./kg.	4	4	6.5	13.7	19.7	22.7	23.5	23.7	23.7
Untreated	16	16	6.7	20.2	37.3	40.7	43.9	44.4	44.4

mals with large numbers of grubs. In heavily infested animals some of the grubs are lost a few days or a few weeks after appearing in the back and do not complete their larval development. In this test grubs that remained in the back less than 4 weeks were excluded from the counts. Therefore, only the counts for treated animals include all grubs that encysted in the back whether or not they completed larval development.

A summary of the grub counts is presented in table 1. It will be noted that all the sprayed animals were completely free of grubs except the two that were sprayed with a 0.5% suspension. One of these yearlings had seven grubs and the other had one. The single grub was present in the back for 2 to 4 weeks, but it did not complete its development. All the untreated cattle were infested with both species of cattle grubs. The average number was 44.4 per animal, and the range from 8 to 108, only three animals having less than 20 grubs.

No explanation is available for the failure to obtain complete control with the 0.5% suspension in view of the results obtained with the 0.25% suspension. Perhaps the winter coat was slightly more water-repellent, perhaps the application was slightly less thorough, or perhaps the absorbed dose was metabolized in a different manner.

The four cattle that received a single oral dose were infested with an average of four grubs per animal while those treated daily for 5 days had 24 grubs per animal. Since these animals were treated almost a month later than the sprayed groups, it is possible the difference between the two methods of treatment was due to the timing.

The cooperating veterinarians[2] made clinical observations and determined the blood cholinesterase values of the animals orally treated. Mild symptoms of toxicity and drastic reductions in blood cholinesterase were noted after both oral treatments, and it was therefore concluded that oral treatment of cattle at 25 mg./kg. was impractical. However, spray treatments with Bayer 21/199 appear to be a highly practical method of grub control if they are made at the proper time of the year and in such a manner as to insure adequate dermal absorption of the insecticide. According to the veterinarians (Radeleff *et al.* 1955, 1957, and unpublished work), single applications of a 0.5% spray may be made without harm to cattle. Dairy calves 1 to 2 weeks old are much more susceptible: 0.5% gave 50% cholinesterase inhibition, and 0.75% was a lethal dose.

[2] R. D. Radeleff and George T. Woodard, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture.

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Dow ET-57 as a Fly Larvicide[1]

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ABSTRACT

Field comparisons of Dow ET-57 and malathion for determination of larvicidal efficiency against house flies and other types of Diptera under caged laying hens were made, using surface application of 1¼% solutions. Dow ET-57 gave an overall control of 78 to 99% at the end of the first and second week, respectively, while malathion gave a corresponding 0 to 67% control.

In recent years, many insecticides have been tested for larvicidal activity against house flies. Few of these materials gave control for more than 1 week. Hoffman & Monroe (1956) at Fresno, California, reported that Diazinon, Bayer L 13/59, Am. Cyanamid 4124, Chlorthion, dieldrin, and malathion at various concentrations gave control of house fly larvae in poultry droppings. Reinfestation occurred within 2 to 9 days following these treatments. Mortalities were not greatly affected when the formulations were applied at dosages ranging from 40 to 100 ml. per square foot. Applications of 200 and 300 mg. of malathion gave 30% control. At 500, 1000, and 1500 mg. per square foot, malathion gave 95, 97, and 93% control with reinfestation occurring at 4, 5, and 6 days, respectively, after treatment. Sampson (1956), at Berkeley, California, used 225 ml. of 0.5% malathion liquid per square foot of surface organic matter and reported a 100% mortality of house flies. Lindquist & Fay (1956) reported that malathion was the least effective of eight organic phosphates tested in the laboratory as larvicides against nonresistant house flies. A more recent report by Wilson & Gahan (1957) at Orlando, Florida, showed that malathion at 250, 200, and 100 mg. per square foot gave 92, 95, and 96% control for 2 days, 15, 77, and 73% control for 7 days, and no control at the end of 14 days. They also found that Dow ET-57 was effective against house fly larvae for periods varying from 2 to 7 days.

The tests reported here were conducted to determine the efficacy of Dow ET-57 (Dow Chemical Company), as a larvicide for house flies and other types of Diptera that develop in droppings of caged laying hens. A 25% Dow ET-57 wettable powder was compared with an established insecticide, a 57% emulsifiable concentrate of malathion (American Cyanamid Company). The experiment was conducted in two separate phases during the spring and fall of 1957 at the Gooch Mills' Juniata Ranch, Manhattan, Kansas.

PROCEDURE.—A poultry house (50′×30′) containing two levels of caged laying hens in three double and two single rows was used. Each row was divided into three sections designated as control, Dow ET-57 and malathion, giving a total of eight sections for each treatment. Each section measured 13.3′×2′ and the treatments were arranged so as to minimize tle effect of position and possible spray drift. The upper levels of cages were not treated since they were cleaned weekly to decrease weight on catch pans.

Dow ET-57 and malathion were each tested at a concentration of 1¼%, applied semi-monthly, four consecutive times during spring and three consecutive times during the fall, at the rate of 107 ml. per square foot (1280 mg. and 1398 mg. per square foot, respectively), using a 3-gallon Hudson hand sprayer having a "T" type nozzle.

Manure samples were collected weekly from the bases of two separate cones of droppings under each treated and untreated section using a No. 12 ice cream scoop (approximately ¼ pint). Different cones were sampled each week. To eliminate possible contamination by the adjacent treatment, no samples were collected from a 2-foot area between sections.

The samples were placed in 7-ounce Dixie cups, brought into a rearing room at 80±2° F. and covered with dry Chemical Specialties Manufacturers Association fly-rearing media. Each cup was then set in a large-mouth Mason jar, the top of which was then covered with heavy muslin. The samples were left in the jars for 3 weeks at which time emerged adults were counted and recorded.

Using a Beckman model G pH meter, comparisons were made of the pH of the droppings in the different sections before and after drying had occurred. Random samples of droppings were collected from different areas within the same sections and mixed together. Small aliquots of the moist sample were taken and the pH determined immediately. The rest of the sample was air dried for 3 days, ground to a powder, and 5-gram aliquots were each mixed with 15 ml. distilled water and the pH determined.

At the end of the spring phase of the experiment, since the test was to be discontinued until later in the season, all surface area of the sections, the lower walls of the building, and upper catch pans were sprayed with a 1¼% solution of Dow ET-57 as a practical control measure against flies.

During the 45 days between the termination of the spring phase and the beginning of the fall phase the poultry house was cleaned and disinfected. The fall phase of the experiment was conducted in the same manner as the spring phase with the exception that no pH determinations of the droppings were made.

The purpose of the fall phase of the experiment was to determine any difference in results that might exist between early- and late-season treatments.

RESULTS.—Throughout the 8 weeks of the spring experimental period, Dow ET-57 was superior to malathion in control of fly larvae in chicken droppings (table 1). At the end of the first week following each spraying, Dow ET-57 gave 78 to 99% control compared with malathion's 0 to 25% control. At the conclusion of the second week following each spraying, control by ET-57 ranged from 95 to 99% compared with 3 to 37% control for malathion.

Throughout the 5 weeks of the fall experimental

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Table 1.—Per cent fly mortality after treatment with Dow ET-57 and malathion during the spring of 1957.

Date of Treatment	Weeks After Treatment	Section[a]	House Flies No.	House Flies Per cent Control	No. Other Diptera	Total Diptera No.	Total Diptera Per Cent Control
June 3	1	Control	1		212	213	
		ET-57	0	100	5	5	98
		Malathion	135	0	40	175	18
	2	Control	3		89	92	
		ET-57	1	67	0	1	99
		Malathion	12	0	46	58	37
June 17	1	Control	28		119	147	
		ET-57	3	89	1	4	97
		Malathion	2	93	108	110	25
	2	Control	13		191	204	
		ET-57	2	85	1	3	99
		Malathion	31	0	124	155	24
July 1	1	Control	59		142	201	
		ET-57	0	100	1	2	99
		Malathion	112	0	74	186	7
	2	Control	368		72	440	
		ET-57	22	94	0	22	95
		Malathion	307	17	122	429	3
July 15[b]	1	Control	949		120	1069	
		ET-57	222	77	13	235	78
		Malathion	1086	0	142	1228	0
July 29		ET-57[c]	2		2	4	
		ET-57	1		0	1	
		ET-57	31		0	31	

[a] Replicated eight times.
[b] Spray coverage may have been insufficient due to inexperienced spray operator.
[c] All droppings treated.

Table 2.—Per cent fly mortality after treatment with Dow ET-57 and malathion during the fall of 1957.

Date of Treatment	Weeks After Treatment	Section[a]	House Flies No.	House Flies Per Cent Control	No. Other Diptera	Total Diptera No.	Total Diptera Per Cent Control
Sept. 14	1	Control	1855		3	1858	
		Dow ET-57	45	98	1	46	98
		Malathion[b]	937	50	12	940	49
	2	Control	911		64	975	
		Dow ET-57	21	98	0	21	98
		Malathion[c]					
Sept. 28	1	Control[d]	493		45	538	
		Dow ET-57[d]	12	98	0	12	98
		Malathion	392	20	35	427	31[e]
	2	Control	874		86	960	
		Dow ET-57[d]	40	96	6	46	95[e]
		Malathion	1677	0	60	1737	0
Oct. 12	1	Control	396		125	521	
		Dow ET-57	16	96	0	16	97
		Malathion	169	57	4	173	67

[a] All sections replicated eight times except where indicated.
[b] Sprayed at one-third strength by error.
[c] No sample taken because of insufficient spraying.
[d] Seven replications.
[e] Calculated on average flies per section.

Table 3.—Comparison of average pH of moist and dry chicken manure.

Treatment	Moist	Dry
Untreated	7.57	7.27
Dow ET-57	7.95	7.83
Malathion	8.0	8.28

period, Dow ET-57 was again superior to malathion (table 2), giving 97 to 98% control at the end of the first week compared with 31 to 67% for malathion. At the end of the second week Dow ET-57 gave 95 to 98% control compared with zero for malathion. However, only one sample (eight replicates) was collected at the end of the second week.

Although no unsprayed control areas remained for comparison when all 24 sections were sprayed with Dow ET-57 at the end of the spring experimental period, further successful fly control by this material was indicated because only 36 flies survived from the total 24 sections sampled 1 week later, as compared with 2,532 flies which survived from comparable samples of treated and untreated sections taken the previous week.

The pH readings on the moist droppings (table 3) in the untreated sections averaged 7.57 compared with 7.95 in the sections treated with Dow ET-57 and 8.0 for the malathion-treated sections. The pH readings on the air-dried droppings from the three categories were: untreated, 7.27; Dow ET-57-treated, 7.83; and the malathion-treated, 8.28.

Discussion.—In the present experiment emphasis was placed on the efficacy of Dow ET-57 and malathion as larvicides under certain field conditions. Factors influencing the effectiveness of larvicides, *i.e.*, "amount of moisture in the pile; presence of impervious, shielding or deflecting material; presence of irrigation channels; and the driving-out effect of chemicals involved," as cited by Sampson (1956) were not considered in this investigation. It was noted, however, that the lowest percentage of control for both treatments was found in wet areas in the respective sections, thus indicating that both compounds gave less control under wet conditions.

On July 15, the spraying was done by an inexperienced operator which may account for the somewhat lower than usual control with both insecticides for that date.

The use of malathion as a larvicide gave very erratic control. In one instance when malathion was used at one-third strength (466 mg. per square foot) by mistake, it gave 49% control. The following treatment was at the full 1¼% and gave zero control at the end of 1 week. At the end of the first week of the last treatment, malathion gave a 67% control. The reason for this behavior of malathion remains unknown.

It was also noted throughout the spring experimental period, June 3 to July 29, that there were fewer house flies than other Diptera. The situation was reversed during the fall experimental period, September 14 to October 19, when house flies greatly outnumbered other flies. Whether the low control of flies by malathion was the result of resistant house flies has not been established.

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Biological Control of Rhodes-Grass Scale in Texas[1]

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Abstract

The degree to which *Anagyrus antoninae* Timb. attacks Rhodes-grass scale, *Antonina graminis* (Mask.), in the south Texas area was found to vary considerably throughout the year and at various locations. The parasite could be recovered in a drouth area of King Ranch, Kingsville only after repeated releases during the period, October 1955 to January 1956. At several locations in the Lower Rio Grande Valley, parasitism decreased during April and became progressively less to the point of being scarce during the summer months. The greatest extent of parasitism occurred during October to March. Parasites were more prevalent during periods in which temperatures seldom reached 90° F. The low relative humidity in the summer and fall of 1956 was considered an important factor responsible for the low parasite activity during the fall.

Laboratory tests showed that *Marietta graminicola* acted as a hyperparasite of Rhodes-grass scale through *A. antoninae*.

Several parasites of grass-infesting scale insects were introduced from France, but no recoveries have been made. Several predators of the scale were found.

Several methods of controlling the Rhodes-grass scale, *Antonina graminis* (Maskell), have been investigated. Chemical control of the scale on range grasses has proven impractical due to the cost of applying materials and the difficulty in reaching the insect. Some range grasses have been found nearly immune to attack by the scale and strains of Rhodes grass vary in susceptibility to attack. The biological control approach was begun in 1949; however, the studies, herein reported, began in February 1954.

The work was divided into four different phases of study. A concentrated effort was made to recover from the drouth area of King Ranch, Kingsville, Texas, parasite of the scale from Hawaii which was introduced in 1949, and to investigate the factors that may limit the activity of the parasite. The extent to which the parasite attacked Rhodes-grass scale and the relationship of a possible hyperparasite to the parasite were important considerations. Foreign exploration for parasites of the scale was continued. Predators or possible parasites of the scale found locally were observed from time to time.

Rhodes-grass scale was originally described as *Sphaerococcus graminis* by Maskell (1897) from an undetermined grass at Kowloon, across the bay from Hong Kong on the coast of China. Clancy (1950) reported the scale had been present in Hawaii since 1910. Ferris (1953) reported it from an undetermined grass in Chiriqui Province of Panama. Chada *et al.* (1950) reported the distribution of the scale in Texas, Louisiana and Florida. The first record of the scale in the United States was on November 17, 1942 from Rhodes grass at Kingsville, Texas. Host records of the scale have been given by Chada *et al.* (1950) and Riherd & Chada (1952).

Antonina indica Green, which is now considered a synonym of *graminis*[3], has been reported from several countries of Asia, the Philippines, South Africa, South America and several islands in the Pacific.

Studies with *Anagyrus antoninae* Timberlake.—*A. antoninae* belongs to the Encyrtidae and was described by Timberlake (1920) from specimens reared from *A. graminis* (Mask.) on Bermuda grass from Hawaii. The parasite was thought to have been introduced with its host some years before from the Orient. Several lots of parasitized Rhodes-grass scale, collected from the Honolulu area early in 1948, were sent to Weslaco by Clancy (1950) who reported effective control of the scale by this Encyrtid parasite. Riherd (1951) reported that the parasite was established in the Lower Rio Grande Valley but not in the King Ranch area where the host plants of the scale had suffered severely from the lack of moisture. Riherd (1950) described the life history of the parasite and a laboratory method of propagating it. He reported that most of the larval feeding, if not all, was on the eggs of the host.

A technique was developed to measure percentage parasitism for *A. antoninae*. One or two scale per node of a random-selected sample of infested grass were dissected under the microscope for larvae, pupae and pre-emerged adults. The percentage parasitism and the average number of parasites per parasitized scale was determined. A measure of parasite activity was obtained by multiplying the percentage parasitism by the average number of parasites per parasitized scale. During periods of low parasite activity microscopic dissection did not show parasitism, yet parasites were sometimes reared from the sample of scale-infested grass. Larger samples were then taken so that sufficient material could be left in 1-gallon cylindrical cardboard food containers for emergence. A 20-ml. shell vial was placed in the side of the container to collect the parasites as they emerged and were attracted to the light. A maximum of seven parasite larvae have been dissected from a single scale.

Tests were conducted at Caesar Pens, Laurelles Division of King Ranch, Kingsville to determine if *A. antoninae* could be recovered at this location. The site was ideal in that the Rhodes grass was heavily infested with the scale and would not be grazed by cattle. Also, an adjoining, fenced pasture of Rhodes grass could be sampled to determine if the parasite was further limited by a relationship of the open pasture compared with the Rhodes grass immediately beside a body of water. Cages, 4×4×6 feet covered with 60-mesh plastic screen, were placed over heavily-infested Rhodes grass immediately

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[3] Recent correspondence with Insect Identification and Parasite Introduction Laboratories at Beltsville, Md.

beside a lake. Cages 1 and 2 received 10 and 20 pairs of parasites, respectively, on November 16, 1954 while cages 3 and 4 received 30 and 40 pairs, respectively, on December 12, 1954. Several releases were made outside the cages and in the adjoining pasture. Results of scale dissections made from samples taken inside the cages are shown in table 1. Monthly samples taken by the lake and in the adjoining pasture showed no parasitism. Samples taken in the pasture May 3 and 25 and July 29, 1955 showed no parasitism as did monthly samples taken regularly from November 1955 till July 1957. Monthly samples taken regularly immediately beside the lake from October 1955 through May 1956 also showed no parasitism. A few adults were reared out of these latter samples taken during October and November 1955 and January 1956. It is interesting, however, that these recoveries were made after several releases of laboratory-reared parasites. No recovery was made during the period, October 1956 to July 1957, which was several months after any parasites had been released.

Another sampling site was located near Headquarters, Santa Gertrudis Section, King Ranch. This location was across Santa Gertrudis Creek from a 75-acre pasture which had been reportedly killed out by the scale and drouth. Some of the first releases of the parasite were made in that pasture. Rhodes grass, moderately infested with the scale, was growing on the banks of the stream and next to a trash dump. Releases of parasites were made in February 1956 and regular monthly samples from March 1956 to July 1957 showed no parasitism.

Samples of infested grasses were taken monthly at several locations to determine the extent to which *A. antoninae* attacks Rhodes-grass scale in the Lower Rio Grande Valley. Percentage parasitism was determined by the dissection method. At Hoblitzelle Ranch north of Mercedes, both Para and Johnson grass were sampled. The Para grass grew alongside a lake with good windbreak sheltering the sampling site from the south. This site was very favorable since the lake shore offered a higher relative humidity and some protection from colder weather as well as a seemingly more uniform scale population. Johnson grass was sampled at another portion of the ranch where the grass seldom suffered from lack of moisture. Rhodes grass was sampled in a non-irrigated pasture at Wood's airport just east of Donna. This pasture had been in Rhodes grass for several years, having been cut only on few occasions for hay. Rhodes grass was sampled in an irrigated pasture at Clifford Ranch south of Mercedes. This pasture was subject to heavy rotation grazing and was irrigated when necessary. Another Rhodes grass pasture was sampled at Water's Ranch south of Donna. This pasture was dry most of the time, although two irrigations were applied. Also, it was grazed at irregular intervals. Percentage parasitism of the scale and the average number of parasites per parasitized scale are given in table 2.

Table 1.—Per cent parasitism of Rhodes-grass scale by field cage test conducted at Caesar pens, Laurelles Division, King Ranch, Kingsville, Texas.[a]

Date of Sample (1954–55)	Cage No. 1 (10 ♀ 10 ♂)	Cage No. 2 (20 ♀ 20 ♂)	Cage No. 3 (30 ♀ 30 ♂)	Cage No. 4 (40 ♀ 40 ♂)
Dec. 2	5(1.4)	1(1.0)		
Dec. 20			0	3(2.0)
Jan. 25	6(1.8)	4(3.5)	10(2.5)	0
Feb. 24	0	6(2.2)	1(2.0)	6(2.8)
Nov. 4	0			0

[a] Numbers in parenthesis give average number of parasites per parasitized scale. Samples taken outside cages showed no parasitism.

A sample of scale-infested Bermuda grass taken on the north side of the lake at Hoblitzelle Ranch showed 14% parasitism with 1.9 parasites per parasitized scale on January 10, 1955.

Parasitism of the scale was heavier during October to March. Parasitism then dropped sharply in April, and during the summer months, it was unusual to find any parasitized scale except at the most favorable locations. Following periods of fall rains, parasitism would again show up in the samples.

Hygrothermograph records at Weslaco during 1955 and 1956 showed variations in temperature and humidity that might have been contributing causes for variations in parasite activity. Average temperatures during the month of decreasing parasite numbers were approximately 1 hour per day above 90° F. with more than 5 hours per day above 80° F. The succeeding 4 to 5 months had about twice the number of hours above 80° and 90° F. The average number of hours during which the relative humidity was below 50% during March to November (inclusive) was approximately 6 hours per day for 1956 as compared with 3 hours in 1955. Relative humidities during 1955 were seldom recorded below 40% whereas 20% humidity was recorded on numerous occasions in 1956 with several records of low humidities of 8, 12, 14, 18 and 19%.

The parasite could be easily reared in the laboratory at 75 to 78° F. on field-collected scale during the summer period when activity of the parasite in the field was very low. This indicated that the increasing number of hours per day above 80 and 90° F. had a suppressing effect on parasite activity in the field. The period of greatest scale activity was observed during the summer and fall months. Thus, the lowest parasite activity occurred during the periods of highest scale activity.

Introduction shipments of adult *A. antoninae* were made to Florida and Louisiana. Live parasites received at Houma, Louisiana totaled 1190 in July 1954, 592 in October 1955 and 250 in June 1956. Some 785 live parasites were received at Belle Glade, Florida during July 1954. During November 1954, 510 live parasites were received at Homestead, Florida.

Marietta graminicola TIMBERLAKE:—Records of parasitism and rearing showed this parasite, which belongs to the Eulophidae, present in samples usually during November and becoming more abundant during January and February. Many published records have reported *Marietta* species as primary parasites on various hosts. Dr. B. D. Burks, U.S.D.A. Insect Identification and Parasite Introduction Laboratories, through correspondence stated that he had received specimens of *graminicola* from Hawaii in 1949 and in checking the collection found *graminicola* from Maryland in 1942 and 1948. He considers most, if not all, *Marietta* species as secondary parasites. *M. graminicola* was fairly abundant during February 1956 so laboratory tests were undertaken to determine if *graminicola* acts as a primary or a secondary para-

Table 2.—Per cent parasitism of Rhodes-grass scale by *Anagyrus antoninae* Timb. at various locations in the Lower Rio Grande Valley of Texas.[a]

	Hoblitzelle Ranch Para Grass			Hoblitzelle Ranch Johnson Grass			Wood's Airport Rhodes Grass			Clifford Ranch Rhodes Grass		Water's Ranch Rhodes Grass	
	1955	1956	1957	1955	1956	1957	1955	1956	1957	1956	1957	1956	1957
January	30 (2.8)	14 (2.4)	24 (2.0)	24 (2.0)	11 (1.5)	7 (2.9)		23 (2.4)	8 (2.0)	9 (2.1)	3 (2.0)	0	20 (1.8)
February	22 (2.7)	38 (2.1)	18 (2.5)	—	14 (2.1)	13 (1.8)		17 (2.0)	9 (2.7)	5 (2.4)	7 (2.9)	—	45 (2.5)
March	34 (3.4)	22 (2.0)	29 (2.3)	6 (1.7)	6 (2.5)	0		7 (1.4)	10 (2.3)	5 (2.0)	1 (3.0)	4 (2.5)	30 (2.4)
April	13 (1.5)	8 (3.2)	29 (2.3)	—	0	9 (2.6)	0	3 (1.0)	4 (2.8)	6 (2.0)	10 (1.8)	2 (2.0)	15 (2.1)
May	—	0	15 (2.6)	—	1 (2.0)	2 (2.0)		1 (2.0)	0	0	0	4 (1.8)	18 (2.4)
June	—	1 (1.0)	22 (2.6)	—	0	2 (2.0)	0	0	4 (1.8)	0	0	0	4 (2.2)
July	—	6 (1.3)	2 (2.0)	—	3 (1.3)	12 (2.3)		0	0	0	0	0	0
August	6.5 (1.2)	0		—	2 (2.0)			0		0		0	
September	3 (2.3)	0		—	1 (2.0)		0	0		0		0	
October	12.5 (2.6)	0		28 (1.7)	0			0		0		0	
November	22.5 (2.4)	16 (1.9)		12 (2.3)	3 (1.7)			0		1 (3.0)		4 (1.5)	
December	10 (2.7)	11 (2.2)		14 (1.3)	5 (2.0)			9 (1.7)		1 (1.0)		9 (1.6)	

[a] Numbers in parenthesis give average number of parasites per parasitized scale.

site of *A. graminis*. Results of these tests are shown in table 3.

Field-collected Johnson grass that was infested with the scale was inverted in vermiculite in a pint cylinderical cardboard food container. The pint container was placed in a 1-gallon container and covered by a plate glass pane. Scale was then exposed to the *A. antoninae* parasites for 24 hours. The *graminicola* parasites were introduced at various intervals and removed after 24 hours. *M. graminicola* was also released in a separate cage to determine if it would act as a primary parasite.

M. graminicola was not recovered from any cage into which the parasite was introduced in the absence of *antoninae*, with *antoninae*, or 14 days after *antoninae*. *M. graminicola* was recovered when released 7 days after *antoninae*, indicating it did act as a secondary parasite.

Rearing and Colonizing Introduced Parasites.—The Entomology Research Division of the U.S Department of Agriculture made available parasites from grass-infesting scale insects closely related to *A. graminis*. H. L. Parker, in the lower Rhone Valley of France, collected parasitized *Antonina phragmitis* Mask. during March 1954 and 1956 and *Antonina purpurea* Sign. during 1954, 1955, 1956 and 1957. These were shipped to D. W. Jones of the Parasite Receiving Station at Moorestown, New Jersey.

During March 1954, two Encyrtid parasites, *Xanthoencyrtus phragmitis* Ferr. and *Boucekiella antoninae* (Ferr.) from *A. phragmitis*, were placed in laboratory trials against *A. graminis*. Neither parasite was observed to show any desire to attack the scale and no parasites were produced in the first generation.

Several possible hyperparasites were removed from the shipments in 1954. One female *Marietta picta* (Andre), belonging to the Eulophidae, was removed from rearing of parasitized *A. phragmitis* during February 1954. The Encyrtid parasites, *Cerapterocerus mirabilis* Westur, *Coccidoxenus* sp. and *Cheiloneurus* sp. were removed from the June and July 1954 shipments.

Laboratory rearing of *Anagyrus diversicornis* Mercet from *Antonina purpurea* was attempted on *Antoninae graminis* during May and June 1954. One adult female was produced in the first generation as early as 17 days after scale was exposed to the parasites. However, most emergence occurred from 19 to 23 days after exposure. Fewer adults were produced in this first generation than originally placed with the scale. No second generation individuals were produced. No field releases were made during 1954.

Field releases of *Anagyrus diversicornis* and attempts to rear this parasite by the method as developed for laboratory propagation of *Anagyrus antoninae* on *Antonina graminis* (except under lower humidity conditions) were made during late May and June 1955. The following field releases were made: 40 females and 18 males released around infested Rhodes grass at Wood's airport, east of Donna; 232 released around infested Para grass at Hoblitzelle Ranch, north of Mercedes; and 15 females and 6 males released around infested Rhodes grass at Caesar pens, Laurelles Division, King Ranch, Kingsville. Three *Timberlakia europaea* (Mercet) from *Antonina purpurea* were also released at the latter location. Monthly recovery attempts have been unsuccessful at these locations. Attempts to propagate *diversicornis* from scale on field-collected Johnson grass under lower humidity conditions in the laboratory were unsuccessful.

Results of shipping adult *europaea* and *diversicornis* during 1955 showed the parasites either to live only a short time after emergence or to be poor "shippers," since only 8 of 414 *europaea* and less than 50% of 985 *diversicornis* were received alive at Weslaco. It seemed advisable to place the parasites into the field immediately after emergence. Therefore, introductory material in isolated vials was brought to this station by D. W. Jones of the Moorestown station on May 25, 1956. Parasites emerging from *A. purpurea* were released soon after emerging and feeding on honey. *Cheiloneurus elegans* (Dalm.), considered a secondary parasite, was killed after emerging. Only one *Cheiloneurus* emerged from any one scale. No *Anagyrus diversicornis* emerged after June 4.

Field-releases were made as follows: 43 female and 15 male *A. diversicornis* and 221 female and 29 male *T. europaea* around Rhodes grass at Wood's airport, east of Donna; 5 female and 4 male *diversicornis* and 87 female *europaea* around Para grass at Hoblitzelle Ranch north of Mercedes; and 7 female and 3 male *diversicornis* and 32 female and 4 male *europaea* around Rhodes grass at Laurelles Division, King Ranch, Kingsville. Monthly sampling at these locations yielded no recovery.

The June 4th to 11th emergence of *T. europaea* was used in laboratory cage tests. Heavily-infested Rhodes grass was planted in vermiculite in 18×18 inch screened cages. Three cages received 50, 52 and 14 adults, respectively. There was no emergence after 50 days.

Table 3.—Tests with *Marietta graminicola* Timb. as a hyperparasite of Rhodes-grass scale through *Anagyrus antoninae* Timb. 1956.

Date (Feb.)	Initial Release				First Generation	
	antoninae		*graminicola*		*antoninae*	*graminicola*
	♀	♂	♀	♂		
9	10	10			166	
1			6	6		0
10	11	10			50	2
17			5	5		
13	10	10	5	5	72	0
14	10	10			55	0
27			5	5		

Table 4.—Field releases of *Timberlakia europaea* and *Anagyrus dilversicornis* during 1957.

Location	*T. europaea*		*A. diversicornis*	
	Female	Male	Female	Male
Wood's Airport	5,743	1,077	278	165
Hoblitzelle Ranch	1,751	317	32	8
Clifford Ranch	158	8	36	8
San Benito Grass Plots	372	43	16	3
Lawn—Weslaco	168	39	31	15
Hdq.—King Ranch	733	87	0	0
Total	8,925	1,571	393	199

Platencyrtus parkeri Ferr. adults, which emerged from *Antonina phragmitis*, collected in France were received from the Moorestown Laboratory on April 13, 1956 and 81 adults were released around Para grass at Hoblitzelle Ranch north of Mercedes. Monthly samples taken have not shown recovery. Five females and 3 males were placed in each of three battery jars containing scale-infested Johnson grass. No first generation adults were produced in the laboratory.

Quarantined material from France via the Moorestown station arrived in April 1957. Of the 2751 vials containing individual parasitized *Antonina purpurea* that were received, 853 vials were labeled as containing *Anagyrus diversicornis* while 1898 vials were labeled as containing *T. europaea*. The material was in much better condition than that received in 1956. As many as 33 *europaea* emerged from a single scale. Releases of these parasites are shown in table 4. A recovery has not been taken to date, October 1957. One female *Marietta picta* (Andre) and 64 female and 39 male *Cheiloneurus elegans* (Dalm.) were destroyed after emergence from the scale.

Predators and Possible Parasites.—Riherd & Chada (1952) reported the following predators of Rhodes-grass scale crawlers: adults of *Hyperaspidius vittigero* (Mann.) and *Hyperaspis indulata* (Say), one of the small checkered beetles, *Hydnocera chapini* (Wolc.) and a brown lacewing, *Sympherobius barberi* Bks.

Several other insect predators have been found to feed upon the scale and crawlers. *Hyperaspis punctata* Lec. Coccinellidae, *Chrysopa* sp. *plorabunda* group Chrysopidae, *Lobodiplosis*, sp., prob. new, Itonididae, have been reared to maturity on the scale. *Labidura riparia* (Pallas) Labiduridae has been observed to entirely consume 25 crawlers in a 60-second period.

Balaustium sp. and *Erythraeus* sp., Erythraeidae, were the only predaceous mites actually observed to feed on the scale. The following predaceous mites have been taken from time to time in field samples of scale-infested grasses: *Amblyseius* sp., *Zercoseius* sp. and *Blattisocius keegani* Fox, Phytoseiidae; *Cheyletia wellsi* Baker, Cheyletidae; *Raphignathus* sp., Raphignathidae; *Anystis* sp., Anystidae; and *Cunaxa* sp., Cunaxidae. The following predaceous mites have been found in the parasite-rearing room around Rhodes-grass scale: *Garmania bulbicola* (Oud.), Phytoseiidae; *Androlaelaps* sp., Laelaptidae; *Liroaspes* sp., Liroaspidae; and *Ereynetes* sp., Ereynetidae.

Several Encyrtid parasites were taken at the King Ranch location. One female *Anagyrus graminicolens* Dozier emerged from a sample of Rhodes-grass scale taken October 6, 1954 and proved to be the first record of this species in the United States. During 1955, *Paraphaenodiscus* sp. was taken in January, *Astymachus* sp. in July *Tyndarichus* sp. in October and *Cheiloneurus* sp. in December. The Eulophid parasite, *Tetrastichus* sp. was taken in January 1956.

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The Distribution and Control of Bagworms in Ohio[1]

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Abstract

In recent years bagworms, *Thyridopteryx ephemeraeformis* (Haw.), have apparently moved northward in Ohio. Damage now occurs throughout the state, but tends to appear in cycles. Ornamental arborvitae and juniper are injured most severely. For many years an arsenical spray applied when the larvae are small has been recommended for the control of this insect. Because home owners fail to observe the small bagworms, newer materials were tested in control of the insect in more advanced stages. Dieldrin appeared more effective than lead arsenate and malathion killed most of the full grown larvae even though feeding had stopped.

The earliest publication on bagworms, *Thyridopteryx ephemeraeformis* (Haw.), from the Ohio Agricultural Experiment Station appeared in 1893 as Bulletin No. 51 by F. M. Webster who stated "so many inquires came to me that a full account of its development and injuries seems desirable for the benefit of those who reside in the southern part of the state. . . In Ohio it has been found in Montgomery County east to Washington County; if it has been found north of this I am not aware of it."

Houser discussed bagworms in Bulletin No. 194 published in 1908 and in Bulletin 332 in 1918. In the latter publication he stated "The range of greatest destructiveness in Ohio extends over the southern part of the state. . . . Rarely is the insect observed north of a line drawn east and west through the northern part of Franklin County."

During each of the past 20 years requests for information concerning bagworms and their control have appeared in the correspondence of the Department of Entomology of the Ohio Agricultural Experiment Station. However, a slightly larger number of letters have been received from the northern than from the southern half of the state. During the past 2 years particularly, bagworms have occurred commonly in Tuscarawas, Stark, Summit, and Cuyahoga Counties. Letters concerning them sometimes indicated that the writer had not observed the strange creature previously.

It is evident that the pest either has moved northward in the state or has become acclimated sufficiently to survive there. This seems to confirm a statement made by Haseman in Missouri (1912) that "Its original home was in the South where it is still most abundant, though it has gradually extended its territory northward." It is interesting to note in the Cooperative Economic Insect Report of the U. S. Department of Agriculture that bagworms were reported causing damage in 18 states during 1957. Although most of the states reporting were southern states, others included were Indiana, Illinois, Iowa, New Jersey, and North Dakota.

Sporadic Populations.—Bagworm populations in Ohio vary greatly from year to year. This is indicated both by general observations and by the number of specimens mailed to the Experiment Station for identification. Other investigators have observed this variation. Haseman (1912) stated that "it appears in sudden outbreaks, being destructive for a year or two and then decreasing as its enemies gain the upper hand." A specific example of this was observed in a cemetery in Zanesville. In 1950 many arborvitae trees were infested and an occasional tree was killed. In many others the tops were killed. Although relatively few of the trees were sprayed in 1950 no living larvae could be found in 1951. An examination of a number of the old bags in the spring of 1951 indicated that internal parasites had been primarily responsible for reducing the population, although winter mortality was relatively high and birds had destroyed some of the larvae.

Although bagworms attack a wide variety of plants, most damage occurs in Ohio on ornamental arborvitae and juniper. Relatively few plants are killed, but the ornamental value may be destroyed by killing a portion of the tops of the plants. Hasemen stated "it is probably the most troublesome pest attacking the foliage of trees in Missouri today."

Bagworm larvae grow more rapidly and cause more damage on the Pfitzer-type than on the upright varieties of juniper. Although the latter types may be damaged severely, the insects are often small when they reach the adult stage. This again corresponds with a statement by Haseman (1912) that "some caterpillars will be full grown before others of the same brood are half grown. When choice food is available, they grow more rapidly than when food is less palatable."

Life History.—A short review of the life history of the insect will indicate the period in which insecticides should be effective. Bagworms live over winter in the egg stage inside the bags. In southern Ohio the eggs may hatch during the latter half of May, but in the northern part of the state hatching usually occurs during the first half of June. Each newly hatched larva immediately constructs the bag in which it lives, and the bag is enlarged as the insect grows.

When a larva becomes full grown, it stops feeding and firmly attaches the bag to a twig. The time when this occurs varies from year to year and depends somewhat on the species of plant attacked, but usually occurs during August. Pupation takes place inside the bag and the adult stage occurs approximately 4 weeks after a larva has stopped feeding. Adult females have neither legs nor wings and do not leave the bags until the over-wintering eggs are deposited.

Control with Insecticides.—Sprays containing arsenicals have been recommended for the control of bagworms for many years. Webster stated in 1893 that Paris green "will make short work of the caterpillars, if applied in late June or even early in July." In 1918 Houser recommended spraying with arsenate of lead paste at the rate of 4 pounds in 50 gallons of water in early June when the larvae are small.

A spray containing lead arsenate has constituted the principal control measure recommended in Ohio as well as in many other states since that time. It has usually

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Table 1.—Bagworm control tests with emulsifiable concentrates. August 24, 1956.

Materials in 100 Gallons	Number Larvae and Pupae	Per Cent Dead
Malathion 57%—2 quarts	450	92.7
Parathion 25%—1 quart	422	78.0
Thimet 47.5%—2 quarts	575	61.9
Untreated	765	7.1

proven adequate if used when the bagworm larvae are small. However, home owners seldom observe the insects and their work until the larvae are well grown and have at least partially defoliated trees. Specimens mailed to the Experiment Station with a request for information concerning the nature of the insect and possible control measures commonly arrive late in July and in August. At that time of the year a spray containing lead arsenate is usually inadequate.

In an effort to improve control measures for well grown larvae, control investigations were carried on in 1950, 1956, and 1957. In each case treatments were replicated five times. Each plot consisted of one well grown arborvitae or juniper plant. In 1950 the sprays were applied with a knapsack sprayer, but in later tests a small power sprayer was used. Approximately 1 week after the sprays were applied, all bags were picked from each test plant and cut open to observe the condition of the insect within.

In 1950 sprays were applied in late July and early August when the larvae were relatively large, but still feeding. Lead arsenate at the rate of 4 pounds in 100 gallons of water appeared more effective than parathion, EPN, DDT, or toxaphene at the strength commonly used in control of other pests. Plans were made for more extensive tests in 1951 in a cemetery in Zanesville where damage occurred in 1950, but no infestation appeared in 1951.

In 1956 bagworm injury was again widespread in Ohio and in some localities damage was severe. Three volatile materials were tested at relatively high concentrations in an attempt to kill full grown larvae inside the bags. The work was done in a cemetery at Strasburg in Tuscarawas County. Sprays were applied on August 24 when approximately half of the bags were attached to twigs and an occasional pupa could be found. The data obtained are summarized in table 1.

Even though approximately half of the bagworms had stopped feeding before the sprays were applied, each of the materials used gave a relatively high kill. Malathion proved to be the most effective of the three materials.

In 1957 a more dilute malathion spray was tested in the cemetery at Strasburg and a series of materials was tested on the state hospital grounds at Cambridge. After the results of these tests were obtained, malathion and dieldrin were used again later in the season on the hospital grounds at Cambridge. This was done in an attempt to eliminate the infestation as well as to obtain more information. The data obtained are summarized in table 2.

Table 2.—Bagworm control experiments. 1957.

Materials[a] in 100 Gallons	Number Larvae and Pupae	Per Cent Dead
Strasburg, July 19		
Malathion 57%, EC—1 quart	238	97.5
Untreated	27	14.8
Cambridge, July 24		
Malathion 25% WP—4 pounds	484	81.4
Lead arsenate —4 pounds	385	87.8
Thimet 47.5%, EC —1 quart	594	10.1
Diazinon 25%, WP —4 pounds	623	56.8
Dieldrin 50%, WP —2 pounds	474	92.6
Untreated	476	0.6
Cambridge, August 2		
Malathion 57%, EC—1 quart	642	92.5
Dieldrin 50%, WP —2 pounds	939	81.0
Untreated	199	0.5

[a] EC, emulsifiable concentrate; WP, wettable powder.

In these tests it appeared that the malathion emulsifiable compound was more effective than the wettable powder. However, it should be mentioned that the small power sprayers that were used contained no agitator. For that reason the data obtained on the insecticides used as wettable powders may not represent the maximum effectiveness of the materials although an effort was made to keep the materials in suspension.

The last counts were made on August 15 and at that time 23% of the living insects found on trees that were not sprayed were in the pupal stage. However, on the same date 77% of those taken from the dieldrin plots were in the pupal stage. It seems evident that these were not feeding when the spray was applied. This apparently accounts for the difference in the effectiveness of dieldrin on July 24 and August 2.

The data seem to indicate that dieldrin is somewhat more effective than lead arsenate in killing well grown larvae that are feeding and that malathion will kill most of the full grown larvae even though feeding has stopped.

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The Comparative NPK Nutrition of *Panonychus ulmi* (Koch) and *Tetranychus telarius* (L.) on Apple Trees[1]

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Abstract

Apple trees were grown in nutrient culture in the greenhouse on varying levels of nitrogen and phosphorus supply during two seasons. Females of *Panonychus ulmi* Koch and *Tetranychus telarius* (L.) were cultured on leaves detached from the trees and the resulting progeny were counted. Analyses of foliage were made for total nitrogen, phosphorus and potassium. Analyses of variance were performed on the results of the foliage analyses and mite counts, and tests for correlation coefficient r were made on absorbed elements with populations.

Increasing nitrogen from 20 to 200 p.p.m. effected significant increases in both mite species. Increasing nitrogen to 800 p.p.m. elicited a further increase from *T. telarius* during one season. Both species were positively related with absorbed nitrogen, but only *T. telarius* was significantly correlated. Previously the author found a negative correlation in tomato foliage at levels of absorption about twice that of the apple trees herein reported, when nitrogen supply was increased, although doubling all the elements in the standard solution resulted in doubled mite populations. Absorbed nitrogen in apple foliage was negatively correlated with *T. telarius* population when phosphorus supply was increased. It was found that in apple foliage, unlike tomato foliage, nitrogen and phosphorus evidenced antagonism, particularly when nitrogen supply was increased. A tendency appeared for absorbed phosphorus to be positively correlated with *T. telarius* below 0.20% phosphorus and negatively correlated when percentages somewhat higher were reached in the foliage. It may be concluded that interrelations among the ions and different ranges of absorption can account for seemingly contradictory effects on mite populations.

Individual variability in mite reproduction rates has made it necessary to deal with large populations in order to draw firm conclusions, and wide percentage differences in populations are required in order to result in significant differences. In this respect *T. telarius* has proved to react with more sensitivity and less variability than *P. ulmi* to changes in element supply and absorption.

The tests showed that *T. telarius* populations develop more rapidly than *P. ulmi* given the same conditions. *T. telarius* appears to react favorably to wider ranges of nitrogen absorption and it follows that it would be capable of doing further damage to foliage after some bronzing by *P. ulmi* has occurred.

Considerable interest has been demonstrated by some workers in the area of orchard mites and their relationship with the host. It has become evident that the influence of the nutrition of the host may be an important aspect in the understanding of mite population development in the orchard.

Evaluating nutritional and fungidical sprays on citrus trees, Thompson (1939) noted that the rust mite, *Eriophyes oleivorus* (Ashm.) became more abundant following copper sprays than where these sprays were omitted. Holloway *et al.* (1942) reported similar results with sprays containing zinc or copper on the citrus red mite, *Panonychus citri* McG. Garman & Kennedy (1949) determined that heavy populations of the two-spotted spider mite, *Tetranychus telarius* (L.) resulted from fertilizer treatments on beans and peach trees in sand culture as compared with unfertilized plants or trees. More recently, Hamstead & Gould (1957) reported on the relation of mite populations to seasonal leaf nitrogen levels in apple trees. Their studies involved maintaining a number of trees on various levels of vigor by supplying two to three levels of nitrogen as fertilizer and following the development of populations of (*Metatetranychus*) *Panonychus ulmi* Koch and tetranychid mites through the season. Analyses for various mineral elements were made and correlations were conducted. The authors noted that *P. ulmi* populations were "substantially greater" in plots showing the highest leaf nitrogen, and that the seasonal mite peaks apparently followed closely the seasonal leaf-nitrogen peaks which occurred at the end of June and midJuly. Data were obtained on the tetranychid mites only on one season and indications were that the peak population occurred about 2 weeks after the *P. ulmi* peak, at the time leaf nitrogen was declining.

Cagle (1946, 1949) studied the life histories of the European red mite and the two-spotted spider mite. Both produced 10 generations per year in his insectary at Blacksburg, Virginia, but the *T. telarius* female produced an average of 62.1 eggs during her lifetime while the *P. ulmi* female averaged only 18.1 eggs.

It was the purpose of the present study to compare the nitrogen, phosphorus and potassium nutrition of the European red mite, *Panonychus ulmi* Koch and the two spotted spider mite, *Tetranychus telarius* (L.) under conditions which were controlled insofar as nutrition of the trees and culture of the mites were concerned. Also it was hoped that information gained would help explain the relationship of *P. ulmi* and *T. telarius* populations on apple trees.

Preliminary work done with apple trees growing in artificial culture in the greenhouse disclosed that the trees grew at an even but accelerated rate during the summer months. This phenomenon is in contrast to the quick spurt of growth made by trees in the field in the spring and early summer, after which growth is relatively slow.

Experimental.—*Tree Culture.*—Carefully selected dormant Close apple trees approximately three-quarter inch in diameter and trimmed to a whip about 3 feet long were set in early April in stone jars each containing about 40 pounds of silica sand. This variety has large flat foliage that lends itself perfectly to detached leaf culture of mites. Each tree was equipped with a reservoir of nutrient solution which flooded with enough solution to saturate

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the sand and then drain back into the carboy. Flooding was accomplished twice daily by an automatic system which forced air under low pressure into the carboys. Three levels of nitrogen, providing 20, 200 and 800 p.p.m. were used; three levels of phosphorus, 2, 20 and 100 p.p.m., were also supplied. Potassium, supplied at 200 p.p.m., was not varied. The standard solution or treated control supplied nitrogen, phosphorus and potassium at 200, 20 and 200 p.p.m., respectively. Each treatment was replicated four times. Iron was supplied in the form of ferro-ferric oxide mixed into the top 3 or 4 inches of sand in each jar. Minor elements were also furnished. In 1956 renewal of solutions was on a schedule 4 weeks apart, except for the 800 p.p.m. nitrogen and 100 p.p.m. phosphorus treatments, which were renewed every 2 weeks. The same plan was followed in 1957 except that the 800 p.p.m. treatment was the only one renewed at 2 weeks. Water was added to bring the solution to the desired level on alternate days during the summer months.

Mite Technique.—Two full-sized mature leaves, at least five or six leaves back from the tip, were taken from each tree into the laboratory where detached leaf culture was used in culturing the mites. This technique as set forth (Rodriguez 1953) was modified in that the leaf disks rested on water-saturated filter paper instead of being floated on sucrose solution. Whenever possible, one series of leaf disks was set up with *P. ulmi* while the other series of disks cultured *T. telarius*. This was not always feasible because *P. ulmi* were field-collected while *T. telarius* came from our greenhouse culture. The careful selection for age which has always been practiced in mite nutrition studies in this laboratory was facilitated in *P. ulmi* by the more obvious teneral condition in this species. Five young adult females were introduced to each disk and cultured in an air-conditioned laboratory at 80° F., about 70% relative humidity and a 15-hour photoperiod. The developmental period was 8 days for *T. telarius* and 10 days for *P. ulmi*. Upon termination of the developmental period, the resulting progeny on each series were refrigerated and then counted. The leaf disks showed no more than moderate injury. Four such experiments of two series each were made during each season.

ANALYSES.—*Foliage.*—Foliage samples were taken from each tree from approximately the same position on the twig as the leaves used for detached leaf culture. Samplings were made at various times during the experimental period which extended from June to September, and a composite sample of foliage from each tree was analyzed for total nitrogen, phosphorus, and potassium (Anon. 1940). In preliminary work, analyses of foliage from similar greenhouse-grown trees made at different times during the season did not disclose significant differences in nitrogen within a treatment.

Statistical.—Analysis of variance, single classification, was performed to determine the effect of treatment on the percentages of absorbed elements; on the results of the mite counts, multiple classification to determine the effect of treatment, series, and the interaction of treatment by series was used. The test for correlation coefficient *r* was performed on the results of the analyses for nitrogen, phosphorus and potassium with the mites that developed on the leaf disks from each replicate. For the analysis of variance, the results from the varying levels of both nitrogen and phosphorus were analyzed together; for the *r* test, the results from the varying levels of the two elements were separated. This provided 20 samples in the analysis of variance and 12 pairs of samples in the test for correlation coefficient, the 200 p.p.m. nitrogen plus 20 p.p.m. phosphorus samples being utilized in both tests for *r*.

Table 1.—Average percentage absorption of elements in foliage of apple trees grown in artificial culture in the greenhouse and resulting mite development. 1956.

Supply (P.P.M.)	Per Cent Absorbed Element			Average Mites per Leaf Disk	
	N	P	K	*Panonychus ulmi*	*Tetranychus telarius*
N levels					
20	2.35	0.18	1.81	51	14
200	2.78	0.14	1.08	91	151
800	3.14	0.16	2.03	98	238
P levels					
2	2.80	0.14	1.59	67	215
20	2.78	0.14	1.08	91	151
100	2.80	0.20	1.52	71	163
L.S.D.					
at 1% level	0.27			31	119
at 5% level	0.19	0.06	0.54	23	89

	r values ($r_{.05} = .576$)	
	N Levels	P Levels
% N with *P. ulmi*	+.529	+.058
% P with *P. ulmi*	−.236	−.077
% K with *P. ulmi*	−.072	−.369
% N with *T. telarius*	+.715**	−.700*
% P with *T. telarius*	+.414	+.594*
% K with *T. telarius*	+.092	+.580*

RESULTS.—*Nitrogen Studies.*—In 1956 when nitrogen supply was 20, 200 and 800 p.p.m., total nitrogen absorption ranged from 2.35 to 3.14% (dry weight of foliage basis), absorbed potassium ranged from 1.08 to 2.03% and phosphorus was influenced but not greatly (0.14 to 0.18%) (table 1). The developing progeny of *Panonychus ulmi* virtually doubled when nitrogen supply was increased from 20 to 200 p.p.m. The increase was from 51 to 91 mites per leaf disk. *Tetranychus telarius* developed only 14 mites per leaf disk at 20 p.p.m. nitrogen supply but mites increased more than 10 times when nitrogen supply was increased 10 times. Both the *P. ulmi* and *T. telarius* populations manifested, therefore, highly significant increases when nitrogen supply was increased from 20 to 200 p.p.m. (table 1). Also it was apparent that supplying more than 200 p.p.m. was of little benefit in boosting *P. ulmi* increases, but of more realistic benefit to *T. telarius* development. The effect of nitrogen supply and absorption on mite development is illustrated in figure 1. In the test for correlation coefficient, a highly significant positive correlation was demonstrated by absorbed nitrogen and *T. telarius* development (table 1). *P. ulmi* manifested strong tendency toward a positive correlation but this was somewhat short of significance.

In 1957, nitrogen supply was identical to the previous year but absorbed nitrogen was lower for all levels of supply. However, the greatest difference in the range of absorption was in 1957, when the absorbed nitrogen ranged from 1.95 to 2.78%. This was particularly noticeable when only the 20 and 200 p.p.m. supply levels were considered, the differences being 0.43 and 0.72% for 1956 and 1957, respectively. Phosphorus absorption decreased significantly at these levels of nitrogen supply, thus an antagonism between these elements which had been less

Table 2.—Average percentage absorption of elements in foliage of apple trees grown in artificial culture in the greenhouse and resulting mite development. 1957.

SUPPLY (P.P.M.)	PER CENT ABSORBED ELEMENT			AVERAGE MITES PER LEAF DISK	
	N	P	K	*Panonychus ulmi*	*Tetranychus telarius*
N levels					
20	1.95	0.36	3.10	84	143
200	2.67	0.17	2.78	114	285
800	2.78	0.17	3.81	98	201
P levels					
2	2.37	0.09	3.00	129	358
20	2.67	0.17	2.78	114	285
100	2.73	0.24	2.96	98	245
L.S.D.					
at 1% level	0.46	0.11	0.62		
at 5% level	0.33	0.08	0.45	34	120

	r values ($r_{.05} = .576$)	
	N Levels	P Levels
% N with *P. ulmi*	+.324	−.302
% P with *P. ulmi*	−.369	−.463
% K with *P. ulmi*	−.151	+.315
% N with *T. telarius*	+.168	−.578*
% P with *T. telarius*	−.431	−.506
% K with *T. telarius*	−.175	+.442

evident in 1956 was well defined in 1957. The same antagonism was manifested in absorbed potassium, more strongly in 1956 (tables 1 and 2). The effect of nitrogen supply on absorption of the elements and on mite populations is illustrated in figure 3. *P. ulmi* increased substantially, approaching significance when nitrogen was increased from 20 to 200 p.p.m., and *T. telarius* increased significantly at these levels (table 2). However, both species decreased when nitrogen supply was increased to the 800 p.p.m. level; this was especially noticeable in *T. telarius*. A comparison of tables 1 and 2 (1956 and 1957) shows that absorbed elements resulting from 800 p.p.m. nitrogen supply was somewhat different. Absorbed nitrogen failed to show a significant correlation with *T. telarius* in 1957 as it had in 1956, although there was a positive relationship between the two in the trees grown at 20 and 200 p.p.m. None of the other elements was significantly correlated with populations of either species.

Phosphorus Studies.— In 1956, increasing the phosphorus supply apparently did not influence nitrogen absorption but it increased phosphorus absorption significantly, though variably, when supply was increased to 100 p.p.m. Potassium was depressed at the standard level (20 p.p.m.) of phosphorus supply. *P. ulmi* development increased significantly at this level when compared to the lowest supply level (2 p.p.m.). *T. telarius* development averaged 215 mites per leaf disk at this lowest level of phosphorus supply, a figure not significantly different from the 151 mites per leaf which developed from the standard level. The test for correlation coefficient showed that absorbed nitrogen was negatively correlated with *T. telarius* while per cent phosphorus and potassium were positively correlated (table 1). The influence of phosphorus supply and absorption on mite development is illustrated in figure 2.

In 1957, increasing phosphorus supply increased nitrogen significantly when 2 and 100 p.p.m. supply levels are considered. Phosphorus also was absorbed in significantly different amounts (table 2). It was again apparent that absorbed potassium was antagonistic to nitrogen at the 2 and 20 p.p.m. supply levels of phosphorus. *P. ulmi* and *T. telarius* on the whole were decreased as phosphorus

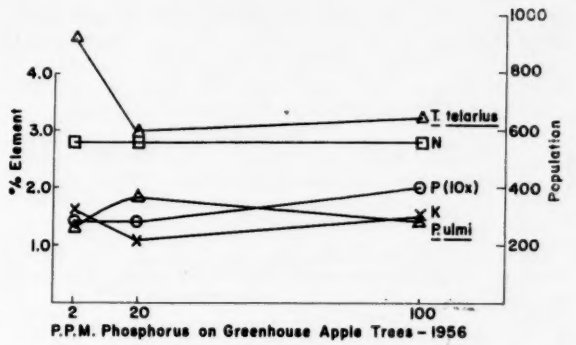

FIG. 2.—The effect of phosphorus supply on NPK absorption and resulting reaction on *Tetranychus telarius* (L.) and *Panonychus ulmi* Koch development. Population is given as total number of mites developing on four replicates (leaf disks). Phosphorus is given as 10 times the actual amount.

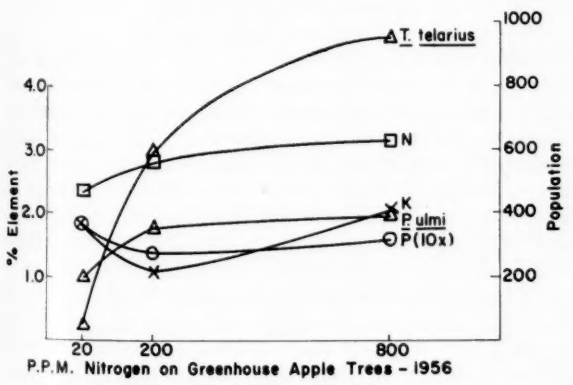

FIG. 1.—The effect of nitrogen supply on NPK absorption and resulting reaction on *Tetranychus telarius* (L.) and *Panonychus ulmi* Koch development. Population is given as total number of mites developing on four replicates (leaf disks). Phosphorus is given as 10 times the actual amount.

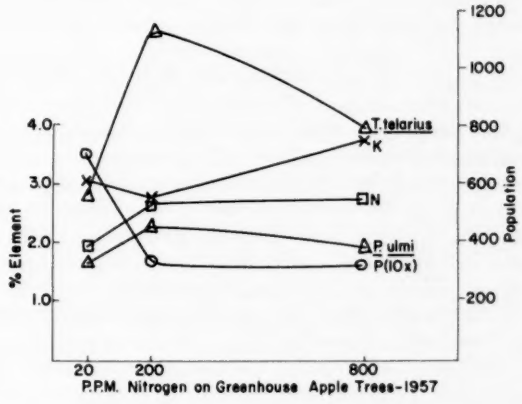

FIG. 3.—The effect of nitrogen supply on NPK absorption and resulting reaction on *Tetranychus telarius* (L.) and *Panonychus ulmi* Koch development. Population is given as total number of mites developing on four replicates (leaf disks). Phosphorus is given as 10 times the actual amount.

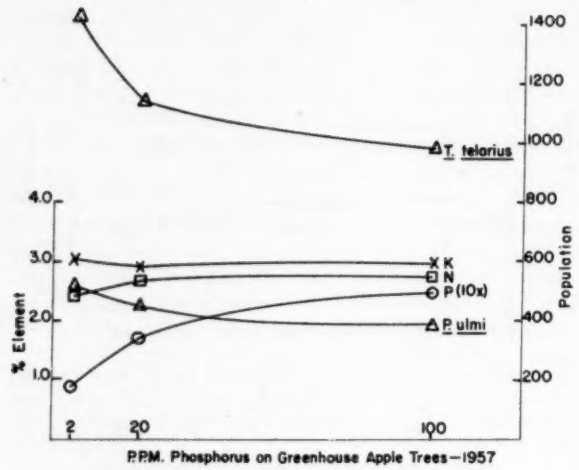

FIG. 4.—The effect of phosphorus supply on NPK absorption and resulting reaction on *Tetranychus telarius* (L.) and *Panonychus ulmi* Koch development. Population is given as total number of mites developing on four replicates (leaf disks). Phosphorus is given as 10 times the actual amount.

increased. In the test for correlation coefficient, absorbed nitrogen was negatively correlated with *T. telarius* population. There was a strong tendency for absorbed phosphorus to be negatively correlated with *T. telarius* development. This will be discussed in more detail later. The relationship of phosphorus supply with the other factors studied is illustrated in figure 4.

DISCUSSION.—In evaluating the evidence presented here, it must be realized that nitrogen supply must affect other plant constituents which may not necessarily be correlated with nitrogen, phosphorus or potassium absorption. Absorbed nitrogen, however, is the element that has more often been associated with increases in mite development. In a previous study the author (1951), working with mites on tomatoes, found a negative correlation existing between absorbed nitrogen and *T. (bimaculatus) telarius*. However, analyses of the tomato foliage disclosed that absorbed nitrogen ranged from 3.16 to 6.82%, generally twice the total nitrogen found in apple foliage grown at the same supply levels. Also, nitrogen and phosphorus were absorbed concomitantly in the tomato foliage, while in the apple foliage antagonism was evident. The absorption range of phosphorus in tomatoes (0.078 to 1.96%) was such that below 0.30% phosphorus the correlation was positive with *T. telarius* population but in ranges above 0.30 the correlation was negative. The tendencies were similar in this respect in the present apple study. The correlation of absorbed phosphorus with *T. telarius* in 1956 was positive; due to variable absorption of phosphorus in the individual trees in the 1956 phosphorus study this effect is not immediately evident in the mean populations shown in figure 2. Per cent phosphorus ranged from 0.10 to 0.21. It has been demonstrated that phosphorus utilization in this mite is relatively heavy (Rodriguez 1954). However, at the wider range in 1957 (0.09 to 0.28) a negative relationship was manifested, especially in the trees containing more than 0.20% phosphorus. On varying levels of nitrogen, somewhat similar changes in range of phosphorus percentages (0.10 to 0.21 in 1956, 0.15 to 0.47 in 1957) and in relationships with *T. telarius* populations (positive in 1956, negative in 1957) existed also in these trees, but here neither of the *r* values was significant. It also must be brought out that the effect of doubling the concentration of the standard solution supplied to the tomatoes was also to double the resulting populations (Rodriguez 1951). Bondarenko (1949) found that the introduction of high rates of mineral fertilizers to hothouse cucumbers increased the osmotic pressure of plant sap two to three times that of normal and also increased *Tetranychus urticae* Koch to high numbers.

Water not being a limiting factor, it follows that turgor pressure may also be increased, although under such conditions, carbohydrates most likely accumulate in the leaf. Nevertheless, this increased turgor pressure introduces the interesting possibility of mites not only obtaining more favorable nutrients but also their feeding being facilitated by such a condition, since in aphids turgor pressure is utilized in feeding (Kennedy & Mittler 1953).

As far as our work is concerned, seemingly contradictory results can largely be explained on the basis of interrelations among the ions and the levels at which they are absorbed. To be sure, the variability of reproduction rates between individual mites has been the source of much difficulty in interpreting results in this laboratory and elsewhere (Garman 1957). Rather wide percentage differences in populations are usually required in order to result in statistical significance. *T. telarius* proved to be less variable in this respect than *P. ulmi*.

The results demonstrated the relationship of *P. ulmi* and *T. telarius* with absorbed nitrogen. Both species increased significantly, although *P. ulmi* increase was just short of significance in 1957, when nitrogen supply was increased (tables 1 and 2). It has been observed in many sections of the United States that natural populations of *P. ulmi* increase rapidly in the early part of the growing season in orchards, while *T. telarius* populations build up several weeks later. Hamstead & Gould (1957) noted that tetranychid mites reached a peak at which time leaf nitrogen was comparatively high but was declining. With the clover mite, *Bryobia praetiosa* Koch on "new" and "old" apple leaves having an age difference of 40 to 50 days and 2.14 and 1.40% nitrogen respectively, Snetsinger (1957) found that females feeding on the higher nitrogen leaves laid an average of 2.37 eggs compared to 0.26 eggs when reared on the lower nitrogen leaves. The studies of Lienk & Chapman (1951) and Chapman *et al.* (1952) suggest that competition of the European red mite may be an important factor in the early suppression and late build-up of *T. telarius*. They also observed, however, that *T. telarius* may build up late even in the absence of *P. ulmi*. Since *T. telarius* is polyphagous whereas *P. ulmi* in contrast is oligophagous it might be expected that *T. telarius* could react to a wider and more general range of nutritional factors than *P. ulmi*. The population curves shown in figures 1 and 3 support this assumption; population trends were similar, but *T. telarius* responded with more sensitivity than *P. ulmi* to changes in nutrient supply. In Kentucky orchards the author has observed populations of *P. ulmi* suppressed rather abruptly by rapid development of *T. telarius* populations in early to midJuly. This

agrees with the findings of Hamstead & Gould (1957) who also suggest the possibility of nitrogen decline being caused not only by the season but also by the effect of feeding of *P. ulmi* populations. Several European workers quoted by Lees (1953) have shown that some winter females of *Panonychus ulmi* may develop prematurely in the second or third generation if severe bronzing occurs early in the season. This premature diapausing condition of *P. ulmi* would leave the foliage free for *T. telarius* attack, providing that the reduced nutrient content of the severely bronzed foliage would support a *T. telarius* population. Premature diapausing of this type in *P. ulmi* evidently has not been reported in the United States (probably because of relatively high summer temperatures), but it has been observed that *T. telarius* does not develop into large populations after the foliage has been severely bronzed by *P. ulmi* feeding. However, *T. telarius* populations are capable of doing further damage to foliage after light bronzing earlier in the season by *P. ulmi*. This is particularly true in the general region of Kentucky, southern Indiana and southern Illinois where *T. telarius* is often the more damaging species (Hamilton 1957). It is interesting to note that in this area this species overwinters on the tree trunk as well as on cover under the tree. That portion of the population that overwinters on the tree trunk evidently moves to the ground cover in early spring where they remain until about late June when they start to move up to the apple foliage.

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Changes in Common Names of Insecticides

A list of common names of insecticides approved for use in the publications of the Entomological Society of America was published in the April 1957 issue, Vol. 50, pp. 226–228. The changes listed below have been approved by the Committee on Insecticide Terminology of the Entomological Society of America, and the new names will be used in future publications of the Society.

The name *Malrin* is no longer approved for use as the common name of a mixture containing 1.8% malathion and 3.6% of Perthane. The owner of this trade-marked name has informed the Committee that in the future the name *Malrin* will not be confined to malathion-Perthane combinations. It will therefore be necessary to state the composition of the mixture used.

Committee on Insecticide Terminology,
CARROLL N. SMITH, *Chairman*

NAME TO BE USED	CHEMICAL DEFINITION	NAME IN 1957 LIST AND OTHER DESIGNATIONS THAT HAVE BEEN USED
barthrin	6-chloropiperonyl chrysanthemumate	ENT-21557
dicapthon	O-(2-chloro-4-nitrophenyl)O,O-dimethyl phosphorothioate	Am. Cyanamid 4124
ethion	O,O,O′,O′-tetraethyl S,S′-methylene bisphosphorodithioate	Nialate, Niagara 1240
siglure	sec-butyl 6-methyl-3-cyclohexene-1-carboxylate	ENT-21486
sesamex	2-(2-ethoxyethoxy)ethyl-3,4-methylenedioxyphenyl acetal of acetaldehyde	Sesoxane, ENT-20871

Laboratory Studies on Selective Aphicides Favoring Natural Enemies of the Spotted Alfalfa Aphid[1]

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Abstract

The failure of parathion to give persisting control of the spotted alfalfa aphid, *Therioaphis maculata* (Buck.), in southern California despite satisfactory initial kill is believed due to the destruction of natural enemies. Late larval and pupal stages of internal parasites of *T. maculata* are not all killed by relatively high dosages of the most toxic aphicides tested, but those materials having the most persistent residues destroy the adults upon emergence. Comparison of the toxicities of 13 aphicides to *T. maculata* adults and to *Hippodamia* adults and larvae show that at the LD-95 for the spotted alfalfa aphid, conservation of coccinellids is highest with nicotine sulphate, and decreases in degree with schradan, demeton, Trithion, Phosdrin, pyrethrum extract, TEPP, lindane, BHC, toxaphene, parathion, malathion, and rotenone. Demeton, because of its high aphid toxicity and favorable effect on coccinellids and parasites, is suggested as the most practical material for integrated chemical and biological control of *T. maculata*.

In 1956 it was recognized that parathion was failing to give satisfactory control of the spotted alfalfa aphid, *Therioaphis maculata* (Buck.), on alfalfa in southern California. An increasingly rapid resurgence of the aphid population followed treatment with this material despite satisfactory initial control. This was considered to be the result of destruction of the aphid's natural enemies. On a number of occasions it was observed that where no treatment was applied, coccinellids reduced the aphid population to non-economic levels, and where the coccinellids were eliminated from the fields by parathion treatment, the aphids came back with unusual rapidity. A review of the evidence that similar pest resurgence often follows treatments that destroy the pest's natural enemies has been presented by Ripper (1956). In this review he listed a number of successful attempts to promote complementary chemical and biological control of pests (a) by the use of physiologically selective pesticides (i.e., pesticides more toxic to the pest than to its natural enemies) and (b) by the modification of treatment practices so as to reduce adverse effects on the natural enemies.

In 1957 a laboratory program was undertaken to examine the physiological selectivity properties of aphicides for use against the spotted alfalfa aphid. The program consisted of a laboratory examination of the differential toxicities of insecticides to the aphid and to its principal natural enemies. It was designed to give as much basic information as possible for anticipated field trials concerned with integrated chemical and biological control. It was particularly desirable to orient the laboratory studies along lines that would be of use in a field examination of the concept that reduction of insecticide dosages to a point giving somewhat less than complete aphid destruction might suffice for economic control, provided favorable natural enemy activity was retained. This concept of a reduced degree of chemical pest control, in combination with conservation of natural enemy activity to produce a lower and more stable pest population balance, has been the inferred basis for a number of successfully integrated chemical and biological pest control programs in Canada and in Europe (Lord 1949, Pickett & Patterson 1953, Ripper *et al.* 1951).

Selectivity of Aphicides to Parasites.—With a few exceptions, adults of the delicate internal parasitic Hymenoptera are at least as susceptible to contact insecticides as are their respective hosts. Nearly all adult parasites are therefore destroyed in the course of field insecticide programs in which heavy application or even overdosage is the rule. Naturally, toxicity to the parasitic species is a function of the dosage. Where low dosages suffice for destruction of the pests, a few of the most selective insecticides may at times permit the survival of some adult parasites directly contacted. A direct contact spray test in the laboratory using adult *Praon palitans* Mues. (an internal braconid parasite of *Therioaphis maculata*) illustrates the degree of adult parasite survival that may be expected. Dosages of the three most selective materials tested; namely, schradan, nicotine sulphate solution, and demeton, which destroyed 95% of the aphids within 24 hours after direct spray treatment, killed, respectively, 68%, 78%, and 96% of the adult parasites.

Further tests were run in the laboratory to compare quantitatively the toxicity of field dosages of some aphicides to three recently imported species of internal parasites of *Therioaphis maculata*. Adults of *Praon palitans*, *Trioxys utilis* Mues. [Braconidae], and *Aphelinus semiflavus* How. [Aphelinidae] were confined with day-old residues which had been applied to wax paper by means of precision laboratory spray equipment. The dosages simulated those commonly applied in the field. At the dosages used, the delicate adults were most susceptible to residues of malathion, parathion, and Phosdrin, they were moderately susceptible to residues of lindane, rotenone, toxaphene, and BHC, and were only slightly susceptible to residues of TEPP, demeton, and nicotine sulphate. Schradan was not tested. The order of toxicity on the basis of time required for 50% mortality was as arrayed above. Examination of the responses to day-old residues, as tested in this instance, incorporates in the test a considerable degree of loss of toxicity due to residue degradation. The rate of toxic residue degradation is of great significance in judging the overall selectivity of contact insecticides to internal parasites. Since most materials destroy the delicate adult parasites, the re-entry and subsequent activity of the parasites in a treated area depends principally upon this characteristic of residual toxicity.

[1] Paper No. 1017, University of California Citrus Experiment Station, Riverside, California. Accepted for publication December 23, 1957.

Residue persistence is recognized as being a function of dosage, and there is some evidence that the period required for breaking or the cessation of toxicity against these delicate species is in direct numerical relationship to the dosage applied.

The period of toxic residue persistence of the aphicides assumes additional significance in view of the expected survival of part of the late larval and pupal stage individuals of internal parasites following exposure to various contact insecticides. Froggatt (1905) first reported the exceptional tolerance of late larval and pupal stages of internal parasites of scale insects to HCN. A similar protection of the late larval and pupal stages of *Aphelinus mali* (Haldeman) inside the mummified bodies of *Eriosoma lanigerum* (Hausm.) was reported for nicotine, lime-sulfur, and oil sprays (Noble 1935). A comparable characteristic survival of *Lysiphlebus* spp. inside the host has been observed by the author after treatment of citrus-infesting aphids with demeton.

Test results showing the effect of three aphicide materials on pupae of two species of internal parasites of *Therioaphis maculata* are presented in table 1. This laboratory test indicates that survival of the pupae is a function of dosage applied, a partial kill being attained with the higher dosages. It may reasonably be presumed from the dosages applied in these laboratory tests and from tests involving other contact materials and parasites (author's unpublished data) that with standard field dosages of all current aphicide materials a variable but significant number of parasite pupae will survive direct treatment. In such circumstances the survival of emerging parasites and the consequent preservation of parasite reservoirs will depend principally upon the persistence of toxic residues. Since the period required for development of the aphidophagous multivoltine parasites from prepupae to adults is approximately 1 week, under optimum conditions, this interval is, in general, taken as the maximum allowable toxic residue period compatible with parasite reservoir preservation. This period varies with prevailing temperatures, it being extended by cooler weather.

SELECTIVITY OF APHICIDES TO PREDATORS.—With the exception of the coccinellids, the effective roles of predators of the spotted alfalfa aphid have not yet been accurately determined. Syrphids, which at times are apparently effective destroyers of *Therioaphis maculata*, are very erratic in their occurrence. The roles of *Chrysopa* spp., *Nabis* spp., *Geocoris* spp., and *Orius* spp. are at present largely unknown. The coccinellids are the most efficient predators of *T. maculata* at high host densities, and their efficiency in restraining incipient aphid outbreaks may be inferred from host increases following coccinellid destruction by high dosages of parathion and malathion.

Ripper *et al.* (1951) demonstrated that schradan and nicotine treatments both gave a favorable selective advantage to aphid-feeding coccinellids. Although early reports of adult and larval coccinellid destruction following high field dosages of demeton to alfalfa were reported by van den Bosch *et al.* (1956), subsequent cage tests with this material in the field indicated that nearly complete alfalfa aphid destruction could be attained without injury to coccinellids, provided minimal dosages were applied. With this background, further laboratory study was confined entirely to examining aphicide selectivity to coccinellids.

Table 1.—Relative toxicity of aphicide materials to pupae of two species of internal parasites of *Therioaphis maculata* after direct spray application to mummified host bodies in the laboratory.

MATERIAL	POUNDS ACTUAL TOXICANT/ GAL. WATER	PER CENT MORTALITY OF PARASITES	
		Aphelinus semiflavus	*Praon palitans*
Demeton	0.250	40	68
	.125	7	41
Parathion	0.125	100	100
	.063	41	84
	.031	15	70
Malathion	1.000	100	100
	.500	88	82
	.250	21	88
	.125	0	59
Water (check)		10	20

COCCINELLID SELECTIVITY STUDIES.—*Methods.*—For toxicity tests with *Therioaphis maculata*, laboratory stocks of a strain of aphid nonresistant to parathion were maintained in a greenhouse on alfalfa. In rare cases these stocks were augmented by field-collected aphids known to be nonresistant to parathion. For adult coccinellid tests, aestivating adults of *Hippodamia quinquesignata* (Kirby) were collected from the field. For larval tests, field-collected larvae of *Hippodamia convergens* Guér. were used.[2]

In tests of the aphids, 50 to 100 adults per unit test were placed on alfalfa bouquets on a turntable in the laboratory and sprayed with mist atomization equipment. Deposit was standardized, and decreasing half dilutions of the aphicide covered the toxicity range. The treated aphid-infested bouquets, kept turgid by stem immersion in water vials, were held in quart ice cream cartons covered on each end with organdie cloth. A light air flow was directed through the cartons with an electric fan for a 24-hour period; mortality counts were then made. Compensation for natural mortality as evidenced by check tests was made by Abbott's (1925) formula, and eye-fitted log-probit curves were drawn.

Squat half-pint ice cream cartons with organdie tops and bottoms were used as containers during treatment and for holding the 40 to 50 adult *Hippodamia* per unit test. Temporary removal of the organdie top of the containers permitted exposure of the lady beetles to a direct-contact aphicide mist comparable to that applied to the aphids. Similarly, half-dilution dosages covered the toxicity range. Check-compensated counts after 24 hours supplied data for the eye-fitted log-probit curves. For *Hippodamia* larval tests, 25 to 30 two-thirds-grown field-collected larvae were treated in the same manner as were the adults.

RESULTS.—Eye-fitted log-dosage-probit mortality curves showing the differential responses of adults of *Therioaphis maculata* and *Hippodamia quinquesignata* and of larvae of *Hippodamia convergens* to 12 aphicides are presented in figure 1. The curve for toxaphene, al-

[2] *Hippodamia convergens* larvae were substituted in the tests for those of the closely allied and practically indistinguishable larvae of *H. quinquesignata* because only mixed stocks of the latter species could be obtained by field collection. Responses to aphicides were similar with limited comparisons.

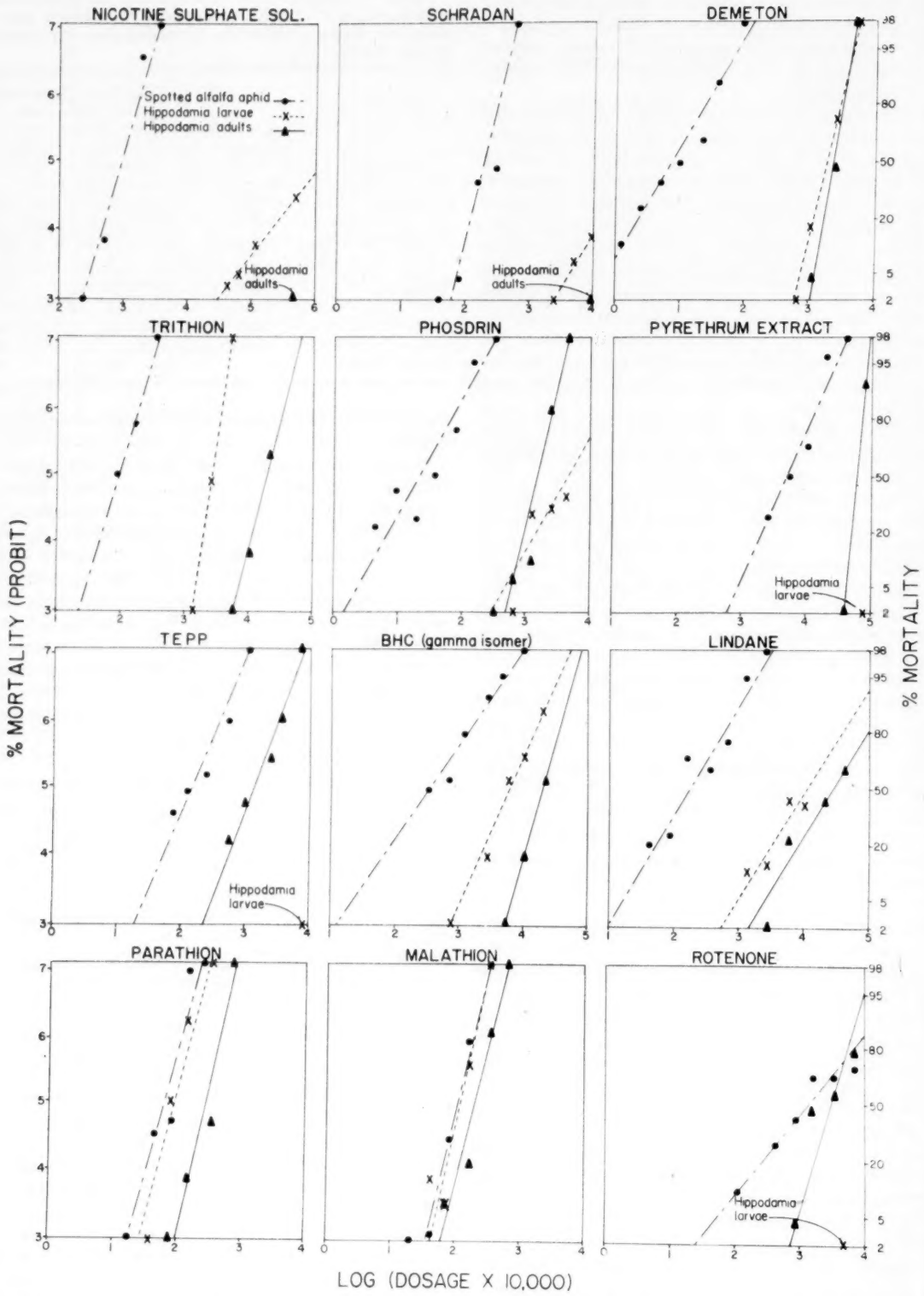

FIG. 1.—Log-dosage-probit mortality curves showing differential toxicity of 12 aphicides to adults of the spotted alfalfa aphid, *Therioaphis maculata*, and to adults and larvae of the coccinellids *Hippodamia quinquesignata* and *H. convergens*, respectively.

Table 2.—**Toxicity of various aphicides to *Therioaphis maculata* (LD-95) and to *Hippodamia* (LD-5), and a calculated value of the selectivity differential favoring *Hippodamia*.**

Material	Dosage Required per 100 Gal.			Selectivity Differential[a] Favoring *Hippodamia*	
	T. maculata	*Hippodamia* LD-5			
	Adults LD-95	Adults of *H. quinquesignata*	Larvae of *H. convergens*	Adults of *H. quinquesignata*	Larvae of *H. convergens*
1. Nicotine sulphate, 40% sol.	0.2 pt.	>48.0 pt.	9.0 pt.	>240.0	>45.0
2. Schradan (actual)	0.05 lb.	>1.0 lb.	0.45 lb.	> 20.0	9.0
3. Demeton (actual)	0.007 lb.	0.15 lb.	0.07 lb.	21.4	10.0
4. Trithion (actual)	0.025 lb.	0.63 lb.	0.15 lb.	25.2	6.0
5. Phosdrin (actual)	0.02 oz.	0.06 oz.	0.045 oz.	3.0	2.3
6. Pyrethrum extract[b]	2.7 pt.	4.0 pt.	>8.0 pt.	1.5	> 3.0
7. TEPP (actual)	0.08 pt.	0.03 pt.	>0.8 pt.	− 2.7	>10.0
8. Lindane (actual)	0.17 lb.	0.25 lb.	0.1 lb.	1.5	− 1.7
9. BHC (actual gamma)	0.5 lb.	0.6 lb.	0.12 lb.	1.2	− 4.1
10. Toxaphene (actual)	1.7 lb.	2.4 lb.	0.28 lb.	1.4	− 6.0
11. Parathion (actual)	0.017 lb.	0.012 lb.	0.0035 lb.	− 1.4	− 4.9
12. Malathion (actual)	0.027 lb.	0.0076 lb.	0.005 lb.	− 3.6	− 5.4
13. Rotenone (actual)	>0.64 lb.	0.09 lb.	>0.64 lb.		

[a] A factorial differential in the dosages, indicating how many times higher the dosage for 5% *Hippodamia* kill is than that for 95% aphid kill (or vice-versa where negative values are expressed), gives a measure of the selectivity of the aphicide.
[b] Pyrethrins, 2.5%.

though not presented in figure 1, is considered in table 2 and in the discussion of results. In table 2 the LD-95 for *T. maculata* and the LD-5 for *Hippodamia* adults and larvae are calculated for each aphicide tested. The order of array of the aphicides as tabulated is, in general, that of decreasing favorable selectivity to both coccinellid adults and larvae. In table 2 the last two columns represent calculated measurements of coccinellid selectivity for adults and larvae, respectively. The figures given represent the number of times that an LD-95 for the aphids had to be increased by the factor of 1 before the LD-5 for the coccinellids was reached. This is given as a positive factorial difference in dosage. Where the LD-5 for the coccinellids had to be increased to obtain the LD-95 for the aphids, the factor is expressed as a negative value.[3] The calculated ratios are considered the best obtainable measures of selectivity favoring the coccinellids. In the table, where it was not possible to obtain 5% destruction of either adult or larval coccinellids within the bounds of usable dosages, it has been necessary to present the selectivity ratio as a figure greater than (>) a certain minimum value. Since with rotenone it was impossible to obtain either 95% aphid kill or 5% kill of coccinellid larvae, only an unmeasurable negative selectivity to adult coccinellids may be recognized.

DISCUSSION.—In some of the curves presented in figure 1 the high mortality sections of the curves were not completed because impractical dosages were required. Also, in some of the curves there was a poor fit to a straight line obtained with log-probit coordinates. It is possible that the fit would have been improved had it been possible to use a more extended aphid count period without high natural mortality. However, extension of holding periods to 48 hours for special rotenone and BHC tests indicated very little increased toxicity other than that associated with increased natural mortality in the checks. Under field conditions a more extended toxicity period for aphids, and possibly for coccinellids, with some materials might well change the selectivity values as here determined. The relatively lower slopes of the demeton and Phosdrin aphid-toxicity curves indicate that it may be possible to approach closer to a desired 95% aphid kill in the field with these materials than with others. Rotenone was the only material used with which there was an indication that high dosages might be more toxic to coccinellid adults than to the aphids, although with malathion and parathion, toxicity to aphids barely exceeded that to coccinellids, particularly the larval stages. The highest dosages of rotenone, TEPP, and pyrethrum used in these tests did not kill the coccinellid larvae, although they did kill the adult lady beetles. Conversely, the coccinellid adults were immune to the highest dosages of schradan and nicotine sulphate solution used, although these same dosages showed a slight toxicity to the larval lady beetles tested.

With respect to the aphid toxicity of the materials as they were tested in this experiment, Phosdrin was the most toxic material at the 95% kill point. It was followed by demeton, parathion, Trithion, malathion, schradan, TEPP, lindane, nicotine sulphate solution, BHC, toxaphene, and pyrethrum extract, in the order given. Rotenone could not be evaluated because of the high dosages required for 95% aphid destruction. In assessing the toxicity of these materials to aphids, possible systemic action was ignored, since field dosages on alfalfa would be expected to be so low that no measurable systemic action would occur.

Examination of the materials in table 2 from the standpoint of selectivity to coccinellids reveals that the first four materials listed show an outstanding degree of favorable selective toxicity. With the fifth material, Phosdrin, it might be somewhat difficult to avoid over 5% coccinellid destruction. This is especially the case when one considers that overdosage is the rule in field applications. Of

[3] This method of calculating selective dosage differentials, despite its change in mathematical base from positive to negative values, appears to the author less confusing and more informative than the decimals obtained by retaining a similar mathematical base.

the four most favorable materials, nicotine sulphate solution and schradan dosage requirements are so high that their use on alfalfa is not economical. Trithion offers some possibility for use where only coccinellids are to be considered, but because of the recognized persistence of this material, preservation of internal parasitic Hymenoptera would be unlikely. Demeton was the most promising selective toxicant from the standpoint of economics and residue persistence. Nicotine sulphate solution was correspondingly the most promising nonphosphate material tested.

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Spray Combinations for Control of the Codling Moth and the Red-Banded Leaf Roller on Apple[1]

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ABSTRACT

Eleven insecticide treatments, including the checks, were tested on apple in combination with the fungicides captan and glyodin, respectively, as control agents for the codling moth, *Carpocapsa pomonella* (L.), and the red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.). With both fungicides, several insecticide treatments controlled the codling moth better than DDT. Also, with both fungicides, Guthion and Sevin (N-methyl-1-naphthyl carbamate) controlled the red-banded leaf roller as well as TDE. Glyodin-insecticide combinations gave better control of both insects than captan-insecticide combinations in most cases.

In southern Pennsylvania, the codling moth, *Carpocapsa pomonella* (L.), and the red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.), attack apple fruits during almost the whole period from fruit formation to harvest. In view of this situation, insecticides or combinations of insecticides that could be applied to control both pests at the same time would have considerable practical value. Also, information concerning the effect of commonly used fungicides on the effectiveness of insecticides against these pests would help give a better understanding of the control problem.

With these points in mind, the experiment reported in this paper was designed: (1) to test certain insecticides or combinations of insecticides as control agents for the codling moth and the red-banded leaf roller; (2) to compare the effect of the fungicides, captan and glyodin, respectively, on the effectiveness of those insecticides and combinations of insecticides against the two pests.

PROCEDURE.—The experiment was conducted in a 5-year-old apple orchard on the Laboratory's Biglerville farm. In all the rows of trees in this orchard, half of the trees are Golden Delicious. Most of the alternate trees are York Imperial, but a few are Rome Beauty. All the data from this experiment were taken from the Golden Delicious trees.

The orchard was divided into 88 plots of six trees each, three trees in each plot being Golden Delicious. This permitted 22 treatments, each replicated four times. In the test, 11 insecticide treatments, including the checks, were applied with captan and with glyodin in parallel plots. As far as the insecticides were concerned, the replicates were randomized throughout the orchard. In all cases, however, the specific captan-insecticide and glyodin-insecticide combinations were applied to parallel plots.

Details of the insecticide treatments are given in table 1. Also, in table 1 under the headings "Codling Moth Entries" and "Leaf Roller Injured," there is one column headed "Captan" and another headed "Glyodin." In each of the six applications of spray, all the plots represented in the "Captan" columns were treated with 1½ pounds of captan per 100 gallons in combination with the insecticide or insecticides listed in the first column. At the same time, each plot represented in the "Glyodin" columns was treated with 1 pint of glyodin per 100 gallons in combination with the respective insecticide treatment. This concentration of glyodin is weaker than is used for most disease control problems on apple, but it is the strongest concentration that will not seriously russet Golden Delicious apples in most seasons.

The sprays were applied at 2-week intervals beginning June 3 and ending August 13, 1957. This timing of sprays was chosen to test the effectiveness of the insecticides in protecting the fruit from injury by second and third

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Table 1.—Results of tests with various combination sprays against the codling moth and red-banded leaf roller at Biglerville, Pa. 1957.

No. of Treatment and Pounds of Materials Used per 100 Gallons[a]	Codling Moth Entries per 100 Apples[b]		Leaf Roller Injured per 100 Apples[b]	
	Captan	*Glyodin*	*Glyodin*	*Glyodin*
1. DDT 50%	28.18	17.28	19.37	12.25
2. DDT 50%, 1.5+Guthion 15%, 1	9.10[c]	5.13[c]	8.72**[d]	4.05**[d]
3. Guthion 15%, 2	7.27*	3.63*	3.63**	3.14**
3A. Guthion 15%, 2+TDE 50%, 1, Aug. 13 only	4.80*	3.63*	4.44**	1.81**
4. DDT 50%, 1.5+RDE 50%, 1	23.19	18.15	7.92**	3.14**
5. Sevin 50%, 2	2.56*	1.81*	7.04**	4.44**
6. Chipman R-6199 75%, 0.5	7.92*	5.74	15.34	10.14**
7. Ethion 25%, 2	6.02*	7.27	17.46	13.31
8. DDT 50%, 1.5+Phosdrin,[e] 1.33 pints	10.78*	10.47	11.39	7.27**
9.[f] Ryania 50%, 2, +lead arsenate, 2, or malathion 25% +TDE 50%	18.72	17.36	12.68	6.29**
10. No insecticide	51.77	78.46	17.05	18.05
	L.S.D. at 1% level	11.93	L.S.D. at 1% level	5.84
	at 5% level	8.98	at 5% level	4.40

[a] Except where otherwise indicated, six applications of wettable powder formulations were applied as follows: June 3, June 17, July 1, July 15, July 29, and August 13.
[b] Expressed as angles of percentages.
[c] Codling moth control significantly better at the 1% level than captan-DDT or glyodin-DDT in their respective groups indicated by one asterisk.
[d] Red-banded leaf roller control significantly better at the 1% level than the captan or glyodin checks in their respective groups indicated by two asterisks.
[e] Phosdrin emulsion concentrate contained 2 pounds of phosdrin per gallon.
[f] Lead arsenate applied in first four sprays; malathion in last two sprays; TDE in last spray only.

generation red-banded leaf roller larvae and also from maximum codling moth activity.

For applying the sprays, two high pressure sprayers equipped with pumps capable of delivering 35 g.p.m. were employed. The pumps were set to deliver spray at 400 p.s.i. at the gun nozzles. By employing two sprayers, it was possible to apply the respective captan-insecticide and glyodin-insecticide treatments to their parallel plots at the same time. Also, this method avoided the possibility of contamination because only one sprayer was used for each mixture.

For collecting data, two Golden Delicious trees were tagged in each plot. All the dropped apples from these trees were scored for codling moth stings and entries and for red-banded leaf roller feeding scars made by second and third generation larvae. In addition, 250 harvested Golden Delicious apples per plot were scored for the same kinds of injuries. Hence, in each treatment listed in table 1 the results are based on 1,000 harvested apples plus all the drops from eight trees. Since the number of stings for the treatments showed the same relationship as the number of entries, these figures were not included in table 1.

The analysis of variance method was employed for indicating the significance of the differences of the results of the treatments. All the figures including those for the checks were used in the analyses of the data recorded on control of the codling moth and the red-banded leaf roller.

RESULTS AND DISCUSSION.—With both captan and glyodin, all the insecticide treatments controlled the codling moth better than the fungicides alone. With captan, all the other insecticide treatments reduced codling moth entries below the number occurring in the apples sprayed with captan-DDT. In the case of seven of these treatments, the differences are significant at the 1% level of probability (table 1). With glyodin, seven insecticide treatments gave better control of the codling moth than glyodin-DDT, four of them significantly so at the 1% level. With both fungicides, outstanding results in codling moth control were obtained with Guthion (alone or in the mixtures with DDT or TDE), Sevin (N-methyl-1-naphthyl carbamate), Chipman R-6199, and Ethion (*O,O,O',O'*-tetraethyl S,S'-methylene biphosphorodithioate).

In all except one treatment (No. 7), the insecticide treatments controlled the codling moth better when combined with glyodin than with captan. However, as far as specific insecticides are concerned only the results of the captan-DDT and glyodin-DDT comparison are significant at even the 5% level. Yet, all the glyodin-insecticide combinations reduced codling moth injuries below the number in the apples sprayed with captan-DDT, seven of them significantly at the 1% level of probability.

From the standpoint of control of the red-banded leaf roller, six insecticide treatments in combination with captan gave results significantly better than the check, five of them at the 1% level. With glyodin, 10 insecticide treatments significantly reduced red-banded leaf roller injury to the fruit, eight of them at the 1% level. Five insecticide treatments in combination with glyodin gave significantly better results in red-banded leaf roller control than when combined with captan, two of them (Nos. 1 and 9) at the 1% level. Two pounds of Guthion wettable powder per 100 gallons of spray in combination with glyodin controlled the red-banded leaf roller significantly better than the captan-DDT-TDE combination. This was the only treatment that was significantly better against this pest than the DDT-TDE insecticide combination with either fungicide.

Two insecticides, Chipman R-6199 and Ethion that gave good results in control of the codling moth performed poorly against the red-banded leaf roller. On the other hand, Guthion and Sevin gave outstanding control of both pests in this test.

Of some importance is the fact that in control of both the codling moth and the red-banded leaf roller glyodin tended to improve the performance of the less effective insecticides to a greater degree than that of the more effective insecticides.

Movement of Insecticides in Soils Under Leaching and Non-Leaching Conditions[1]

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Abstract

In 1954 a Miami silt loam and a muck soil were treated with aldrin at 20 lb. and 200 lb./acre, lindane at 10 lb. and 100 lb./acre and DDT at 10 lb. and 100 lb./acre. The insecticides were sprayed on the soil as an emulsion with a sprinkling can and then rototilled into the soil to a depth of 4 to 5″.

Seventeen months later 84 to 96% of the insecticides were found in the upper 3-inch level of the loam soil, 4 to 12% in the 3 to 6″ layer, and 0 to 5% in the 6 to 9″ layer. In the muck soil 62 to 74% was found in the top layer, 19 to 29% in the 3 to 6″ layer and 7 to 8% in the 6 to 9″ layer. No differences were noticed between individual insecticides. At the same time lindane was found to be unequally distributed in a horizontal direction.

Three years after treatment, slightly (5 to 15°) sloping plots contained from 1.3 to 2.2 times more insecticide in the lower half as compared with the upper half of the plot.

Experiments, conducted under laboratory conditions, showed that lindane was leached to some extent from a treated soil into an untreated one. The leaching was most noticeable in Plainfield sand and least noticeable in muck soil. Under non-leaching conditions, lindane also moved into the untreated layer, but more was retained in a muck soil than in a Plainfield sand. When radioactive parathion (P^{32}) was used, it was found that during a period of 6 days, parathion moved upwards, downwards and sidewards as well. The results obtained seem to indicate that the movement of parathion is more rapid in a Plainfield sand than in a muck soil, as the latter retains the insecticide to a greater extent. In a Plainfield sand, 6.6% of all the parathion recovered was in the untreated layers adjacent to the treated one and 3.5% in the untreated layers most distant from the treated one. The respective figures for a muck soil were 10.8% and 1.8%.

Preliminary experiments conducted with aldrin under non-leaching conditions indicate movement of this insecticide to a considerable extent.

Insecticides from sprayed crops and from direct soil application have been found to persist or even accumulate within soils (Chisholm *et al.* 1951, Fleming *et al.* 1951, Ginsburg & Reed 1954, Ginsburg 1955, Lichtenstein 1957). Such accumulation may be of value in the control of certain soil insect pests but of detrimental value to subsequent crops. Some insecticides may affect the root systems of certain plants and also be translocated into other plant tissues (Simkover & Shenefelt 1951, 1952, Stone *et al.* 1953, Fleming & Maines 1953, Terriere & Ingalsbe 1953, Allen *et al.* 1954, Boswell *et al.* 1955). If insecticides persist mainly in the upper 3 inches of soils, crops with short root systems would be most exposed to those chemicals; if below 3 inches, crops with more extended root systems could be affected. In view of these side effects and for a better understanding of soil insect control, it is of primary importance to define the movement of insecticides within soils. Field and laboratory studies were conducted to measure the movement of some typical insecticides in soils under leaching and non-leaching conditions.

Methods.—*Field studies.*—In 1954, a Miami silt loam and a muck soil were treated with aldrin at 20 and 200 lb./acre, lindane at 10 and 100 lb./acre, and DDT at 10 and 100 lb./acre. The insecticidal applications involved thorough mixing of an emulsifiable concentrate with water. Two-gallon quantities of diluted emulsion were spread as equally as possible with a sprinkling can over successive 10×29 foot areas of each experimental plot (100′×29′). Immediately after application, each plot was rototilled to a depth of 4 to 5 inches.

Seventeen months after treatment, 40 soil cores ($\frac{3}{4}$ inch dia., 9 inches long) were collected from each plot. Each core was divided into three segments (0–3″, 3–6″ and 6–9″), comparable subcores from each plot were mixed and subsequently extracted and analyzed (Schechter & Haller 1945, Schechter & Hornstein 1952, O'Donnell *et al.* 1954, Lichtenstein *et al.* 1956), to determine the vertical distribution of the insecticides.

Certain plots in the loam experiment were used to determine the movement of insecticides down gentle slopes (5 to 15°). Three years after treatment, a 40-core sample was collected in the upper half and a like number in the lower half of four plots (DDT 10 lb./acre, lindane 10 and 100 lb./acre, and aldrin 200 lb./acre). Previously mentioned analytical methods were employed to ascertain the quantity of insecticide in the thoroughly mixed samples.

Laboratory experiments.—Experiments were designed to study the movement of insecticides in different soil types under leaching and non-leaching conditions. One-quart ice cream cartons with perforated bottoms were filled with five to seven cheesecloth separated layers of soil. Columns of Plainfield sand, Miami silt loam and muck soils were used. When lindane was applied, the two upper layers of soil (150 gm. each) were treated at approximately 10 p.p.m. and placed over three untreated layers (150 gm. each). Three variables were employed: 1) No water was poured through the column during a 10-day holding period, 2) 100 ml. of water was poured through each column daily for 5 days, 3) 100 ml. of water was poured through each column daily for 10 days. The watered columns were placed on a Buchner funnel and the water, which passed through, was collected in an Erlenmeyer flask for analysis. After 5 or 10 days the columns were cut open and the different layers carefully separated and then analyzed for lindane content. Results were based on four replicates of each treatment-soil type combination.

To study the movement of an insecticide in soil under

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Table 1.—Vertical distribution of DDT, lindane and aldrin in a loam and a muck soil, 17 months after treatment.

Insecticide	Pounds Applied per Acre	Soil Type	Per Cent Distribution of Recovered Insecticide 0–3″ Layer	3–6″ Layer	6–9″ Layer
DDT	10	Loam	83.6	11.8	4.6
	100	Loam	84.0	11.9	4.1
	10	Muck	66.1	27.1	6.8
Lindane	10	Loam	96.0	4.0	0.0
	100	Loam	87.7	10.0	2.3
	10	Muck	62.3	29.4	8.3
	100	Muck	69.8	24.3	5.9
Aldrin	20	Loam	89.0	8.3	2.7
	200	Loam	88.3	8.8	2.9
	20	Muck	74.0	19.2	6.8
	200	Muck	65.7	26.7	7.6

non-leaching conditions in both vertical and horizontal directions, radioactive parathion (P^{32}) was used and the samples were analyzed by counting with a Geiger-Mueller counter. Preliminary experiments were conducted to see whether similar results could be obtained by direct counting of the soil against counting of a soil extract. When the distribution of radioactive parathion in the various soil layers was compared by the two methods, the results were almost identical. Thus direct soil counting seemed to be the easiest practical method for such studies.

Seven 50-gram layers of soil, separated by cheesecloth, were placed into a 1-quart ice cream carton. The middle or fourth layer which had been previously treated with approximately 200 p.p.m. of radioactive parathion, was placed over three untreated layers and then covered with three additional layers of untreated soil. Four identical columns of each soil type (Plainfield sand, Miami silt loam and muck) were prepared, two of which were placed in a vertical and two in a horizontal position. No water was applied and after 6 days, the columns were opened and the layers were carefully separated and individually mixed. One-gram aliquots which proved to be "infinitely thick" were counted against a standard of the same soil. This standard had been treated with parathion at 200 p.p.m. at the beginning of the experiment.

A partition into chloroform and water was done from an acetone extract, to determine whether the P^{32} recovered within the various soil layers represented parathion (chlorofrom soluble) or nontoxic hydrolyzed products of parathion (water soluble). Thirty grams of each soil layer from a loam column were extracted with warm acetone. After filtering, 5 ml. of water was added to the filtrate, the acetone was evaporated and the amount of water adjusted to 5 ml. in a 15 ml. calibrated centrifuge tube. Five ml. of chloroform were then added and the water-chloroform mixture was shaken thoroughly. After centrifuging, 1-ml. aliquots of the water and chloroform fraction were counted with a Geiger-Mueller counter.

Table 2.—Recoveries of DDT, aldrin and lindane on a sloping loam plot, 3 years after treatment.

Treatment	Degrees Slope (Approximate)	P.P.M. from Upper Half	P.P.M. from Lower Half	Ratio Lower/Upper
DDT, 10 lb./A.	15	1.64±0.02[a]	2.12±0.12	1.29
Lindane, 10 lb./A.	15	0.56±0.03	1.19±0.02	2.13
Aldrin, 200 lb./A.	10	7.92±0.22	17.30±0.20	2.18
Lindane, 100 lb./A.	5	11.2 ±0.10	16.40±0.50	1.47

[a] Standard deviation.

Results.—*Vertical distribution.*—Seventeen months after treatment 84 to 96% of the insecticides (DDT, lindane and aldrin) was found in the upper 3″ layer of a Miami silt loam (table 1). Four to 12% was found in the 3 to 6″ layer and 0 to 5% in the 6 to 9″ layer. In muck 62 to 74% was found in the top layer, 19 to 29% in the 3 to 6″ layer and 7 to 8% in the 6 to 9″ layer. No individual differences between insecticides were observed.

Recoveries of DDT, aldrin and lindane on a sloping plot.—Table 2 summarizes the analytical results of samples collected on the upper and lower halves of four insecticide-treated loam plots located on a gentle slope. From 1.29 to 2.18 more insecticide was found in the lower halves of these plots with DDT showing the smallest difference.

The plot treated with lindane at 10 lb./acre and having a slope of 15° contained 2.13 times more insecticide in the lower half, whereas the plot treated with lindane at 100 lb./acre but having a slope of 5°, contained only 1.47 times more in the lower half than in the upper part of the plot.

Initially after treatment only the upper half had been sampled and analyzed.

Recoveries of lindane from soil columns under leaching and non-leaching conditions.—The results of these experiments (Table 3) seem to incidate that lindane is leached

Table 3.—Vertical distribution of lindane in three soil types under non-leaching and leaching conditions.

Soil Type	Days of Watering (100 Ml./Day)	P.P.M. in Vertical Layers Indicated: Treated 1	Treated 2	Untreated 3	Untreated 4	Untreated 5	Total
Plainfield sand	0	9.65±0.30	9.15±0.40	0.00	0.00	0.00	18.80
	5	7.45±0.77	7.61±0.57	1.01±0.43	0.21±0.08	0.18±0.14	16.26
	10	6.62±0.44	7.98±0.47	1.31±0.12	0.14±0.04	0.06±0.06	16.11
Miami silt loam	0	10.10±0.50	9.52±0.43	0.27±0.00	0.00	0.00	19.89
	5	9.47±0.86	9.55±0.32	0.44±0.10	0.00	0.00	19.46
	10	7.48±0.30	7.44±0.56	0.57±0.09	0.08±0.05	0.08±0.05	15.65
Muck	0	10.55±0.45	10.50±0.20	0.80±0.23	0.16±0.16	0.14±0.14	22.15
	5	9.18±1.44	9.59±1.36	0.09±0.09	0.00	0.00	18.86
	10	9.17±0.07	8.88±0.35	0.15±0.04	0.05±0.03	0.07±0.07	18.32

Table 4.—Distribution (in p.p.m.) of parathion in layers of soil columns under non-leaching conditions, 6 days after treatment.

Soil Layers	Vertical Position			Horizontal Position			P^{32} in Layer Indicated with Vertical Loam Column	
	Plainfield Sand	Miami Silt Loam	Muck	Plainfield Sand	Miami Silt Loam	Muck	Direct Soil Counting	Extract Counting
1. Untreated	6.8± 0.40	4.9±0.65	4.1±0.10	5.0± 0.65	2.3±0.03	4.2±0.43	2.20	2.12
2. Untreated	8.6± 2.30	5.2±0.25	6.5±0.71	6.7± 1.47	2.6±0.62	5.7±0.10	2.15	1.75
3. Untreated	10.7± 0.45	19.9±0.85	25.7±0.85	9.4± 0.20	14.4±0.65	19.1±2.15	9.00	8.50
4. *Treated*	140.0±15.0	164.0±7.00	155.0±3.00	144.5±26.00	161.5±3.50	152.0±1.00	74.00	79.00
5. Untreated	15.4± 2.2	20.4±3.10	23.1±1.10	16.4± 1.00	14.1±1.00	18.0±0.70	9.35	5.60
6. Untreated	9.8± 0.2	4.3±0.65	5.4±0.90	6.3± 0.60	2.5±0.51	5.9±0.15	1.92	1.93
7. Untreated	6.9± 0.6	3.1±0.15	4.1±0.60	6.0± 0.35	4.0±0.27	4.8±0.12	1.38	1.10
Total	198.2	221.8	224.4	194.3	201.4	209.7	100.00	100.00

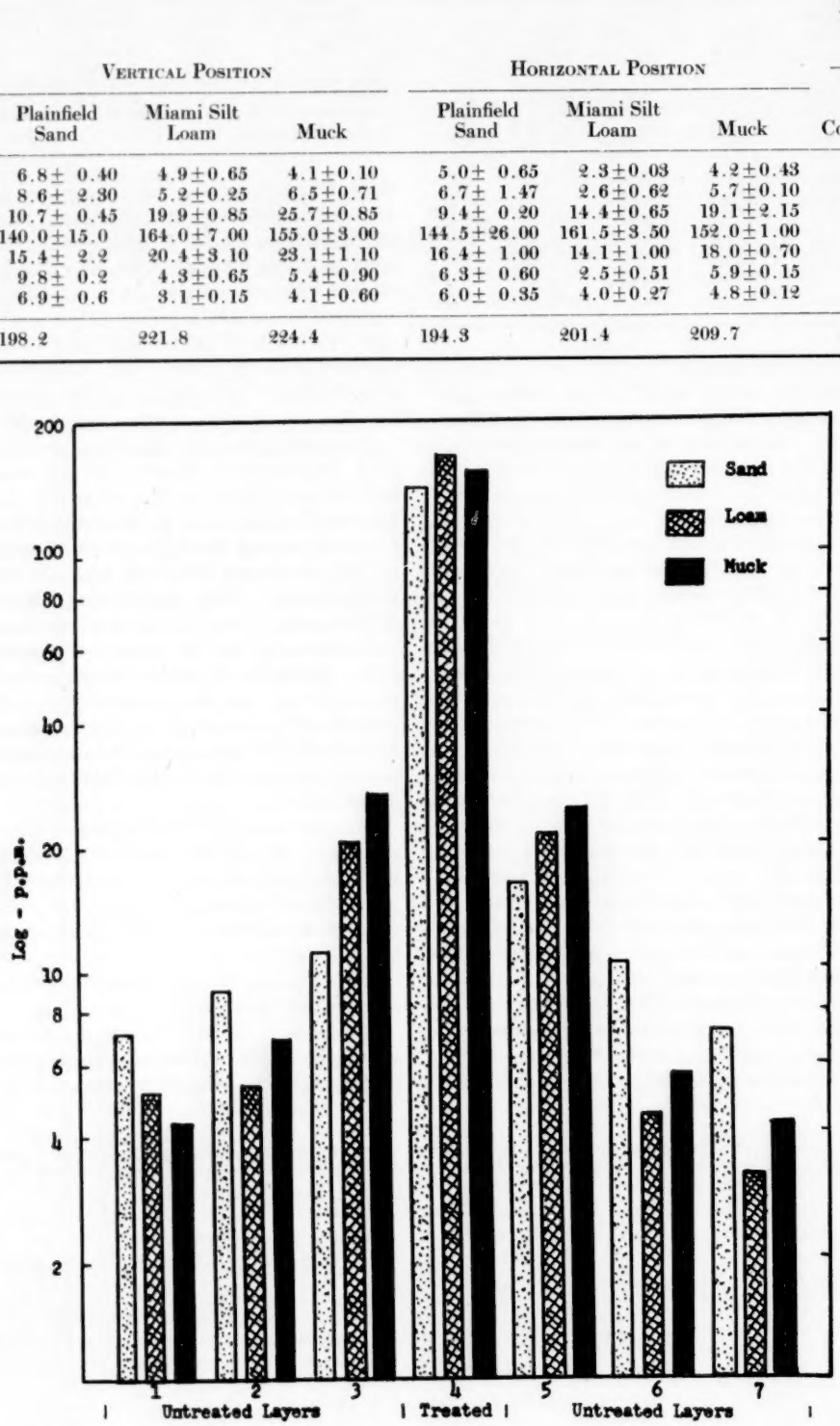

FIG. 1.—Distribution of parathion in layers of soil columns under non-leaching conditions, 6 days after treatment.

into the lower untreated layers (3, 4, 5). The third layer, which is the first untreated one adjacent to the treated layer, contained 1.31 p.p.m. of lindane in Plainfield sand, 0.57 p.p.m. in Miami silt loam and 0.15 p.p.m. in muck after 10 days leaching with 100 ml. water daily. The differences probably are due to a larger retention of the insecticides in soils containing a higher percentage of organic matter. No lindane was found in the water, which passed through the columns and was collected in an Erlenmeyer flask. Under non-leaching conditions, no lindane was found in the third layer of Plainfield sand, 0.27 p.p.m. in the third layer of loam, and 0.80 p.p.m. in the third layer of muck. Moreover the two bottom layers of sand and loam did not contain any lindane, but traces of lindane were found in these layers of the muck soil.

Movement of parathion (P^{32}) in three soil types under non-leaching conditions.—Table 4 summarizes the results obtained on the movement of radioactive parathion (P^{32}) in soils under non-leaching conditions. It was found that parathion moves within the soils in all directions, which seems to indicate a diffusion phenomenon. The layers adjacent to the treated ones contained more parathion in muck (10.8% of all the parathion recovered) than in Plainfield sand (6.6%). The layers farthest from the treated ones contained more in the sand (3.5%) than in either the loam or the muck soils (1.8%). Parathion apparently moves more rapidly in a sandy soil and possibly is lost at the soil-air interface more rapidly than from the other two soil types. The opposite seems to be true in a muck soil (fig. 1).

When water and chloroform fraction of soil extracts were counted, no counts were registered in the water fractions of the two outer layers and only traces in those of the other layers (3, 4 and 5). Therefore it might be assumed that the recoveries given in table 4 and figure 1 actually represent parathion.

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NOTICE TO AUTHORS

On the inside of the front cover of the September, 1957, issue of the *Bulletin of the Entomological Society of America* (Vol. 3, No. 3) the policy of omitting the footnote designating papers for which part of the publication costs are paid (unless authors of such papers requested that it be included) was announced.

At the Annual Meeting held at Memphis, Tenn., Dec. 2–5, 1957, the Governing Board of the Society adopted a resolution requiring that papers published in the *Annals of the Entomological Society of America* and in the JOURNAL OF ECONOMIC ENTOMOLOGY heretofore carrying the footnote "Paid paper" be identified in the future by the following footnote:

"Partial cost of publication of this paper was met by . . . " (Appropriate information on agency, institution, etc., to be inserted.)

The Effect of Some Biologically Active Compounds on Growth of House Fly Ovaries[1]

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ABSTRACT

Fifteen synthetics and three materials of biological origin were tested for their effect on ovarian growth by feeding them to the house fly (*Musca domestica* L.). Coumarin, 1-phenyl-2-thiourea, piperonyl butoxide, *p*-quinone, and thiourea completely inhibited ovarian growth at the concentrations tested, but fish ovarian extract, "queen substance" (a honey bee secretion), and the potassium salt of gibberellic acid had no effect. When injected into the flies, neither "queen substance" nor gibberellic acid showed any effect on growth or as a toxic agent. Fish ovarian extract did not affect viability when applied to newly laid eggs, nor did gibberellic acid cause any change in growth at any stage when introduced in the larval medium.

Attempts to reduce the biotic potential of insect populations by lowering their fertility met with striking success in the work of Bushland & Hopkins (1951) on the screwworm (*Callitroga hominovorax* (Cqrl.)). However, the elimination of entire populations, as was accomplished on the island of Curacao (Baumhover *et al.* 1955) is applicable to few species. Since the uninhibited growth of an insect population is exponential, even a small reduction of its reproductive potential could reduce massive outbreaks of economic pests.

Certain mitotic poisons have been found to inhibit ovarian growth, and consequently oviposition, in the house fly (*Musca domestica* L.) (Mitlin *et al.* 1957). A number of antitumor drugs can accomplish the same effect (Mitlin & Baroody, unpublished data). This paper reports the results of tests of known growth regulators on ovarian growth of this fly. Some are synthetic, others are biologically derived. Although in the house fly only the ovary shows growth, there is no reason why the male gonads might not be affected in other species. The house fly has been used because it is a convenient test insect.

SYNTHETIC MATERIALS.—The synthetic materials and concentrations tested are shown below.

	Mg./ml.		*Mg./ml.*
Coumarin	5	1-Phenyl-2-thiourea	5
Dihydrosafrole	1	Piperonyl butoxide	1
Eugenol	1	*p*-Quinone	5
Guanine hydrochloride	1	Sulfone	1
Hesperidin	0.5	Sulfoxide	1
4-Hydroxyproprophenone	1	Thiourea	2
Maleic acid	1		

The concentrations were of necessity empirical, depending upon the quantities available and the difficulty in formulation. The materials were fed to house flies (1948 NAIDM (National Association of Insecticide and Disinfectant Manufacturers) strain) reared by the CSMA procedure (Chemical Specialties Manufacturing Association 1951). If the test material was soluble, it was dissolved in skimmed milk; if insoluble, the mixture of the material and the milk was hand-homogenized two or three times to make a stable emulsion.

Coumarin, 1-phenyl-2-thiourea, piperonyl butoxide, *p*-quinone, and thiourea completely inhibited ovarian growth, but the others appeared to have no marked effect.

Of the compounds containing the 3,4-methylenedioxyphenyl group, only piperonyl butoxide was effective at the concentrations tested. Compounds containing this group were found generally to inhibit growth when fed to house fly larvae (Mitlin 1956). However, thiourea and coumarin appeared to inhibit ovarian growth as effectively as larval growth (Konecky & Mitlin 1955). The differences are probably due to formulations rather than any basic physiological differences.

BIOLOGICALLY DERIVED MATERIALS.—*Fish Ovarian Extract.*—Heilbrunn & Wilson (1956) reported that extracts from the ovaries of various species of ocean fish had a strong antimitotic action on eggs of the worm *Chaetopterus pergamentacens*. Since ocean species were unavailable, and since specificity was evidently not a factor, we used live goldfish, *Carassius* sp., and sunfish, *Lepomas* sp., to obtain extracts by the method of these authors, substituting artificial sea water in the procedure.

The extracts of three pairs of ovaries of each type of fish were separately incorporated in skimmed milk in equal quantities and fed to newly emerged virgin female house flies by the method of Mitlin *et al.* (1957). To determine the effect of the extract on the viability of house fly eggs, newly laid eggs were allowed to hatch on impregnated filter paper. Neither ovarian growth nor egg viability differed from untreated flies.

"Queen Substance."—This term was used by Butler (1954) to designate a hormone-like material, secreted by the queen honey bee (*Apis mellifera* L.), which inhibits ovarian growth in attendant worker bees. Carlisle & Butler (1956) reported that it is not specific; that it will inhibit ovarian development in worker ants (*Formica fusca* L.), and in the prawn *Leander serratus*. Queen substance has been identified biologically but not chemically.

Twelve mated queens were dipped into 25 ml. of 75% ethanol three times, on alternate days to allow them to recover between dippings. The ethanol rinse was diluted to 50 ml. with water and was used to reconstitute dried skimmed milk. This mixture was tested on newly emerged virgin female house flies in the manner reported (Mitlin *et al.* 1957).

Extract from 12 additional queens was prepared with 25 ml. of 30% ethanol. A dosage of 1.75 μl. per fly was injected into 20 newly emerged female house flies with a microinjector (Dutky & Fest 1942). Preliminary tests had shown higher concentrations of ethanol to be lethal. The flies were held and fed on skimmed milk.

After 3 to 4 days dissected ovaries of the flies were found to have developed as far as those of untreated flies. Evidently under the conditions of our experiment queen substance did not inhibit ovarian growth in house flies.

Queen substance, an intangible material, can be determined only by its biological effects. However, since Carlisle & Butler (1936) did show that it is readily soluble and relatively stable in ethanol we can assume that, in

[1] Accepted for publication January 2, 1958.

pite of negative results, we were working with the hormone.

Gibberellic Acid.[2]—This material has received wide notice recently as a very potent growth accelerator in plants. Derived from the fungus *Gibberella fujukmoi*, it is effective at concentrations of a few parts per million. Although the manufacturer has reported informally that gibberellic acid is not toxic to mice, either orally or intravenously at 2000 mg./kg., very little has been published about its effect on animals.

Tests were made to determine (1) what effect gibberellic acid had on house fly growth, and (2) whether it was toxic to house flies at the concentrations used. A potassium salt of the acid, readily soluble in water and ethanol, was used.

To determine the effect on growth, 450 mg. was added to 450 grams of CSMA medium by the procedure of Mitlin *et al.* (1954). Eggs of the NAIDM strain introduced into the medium hatched, and the larvae had a normal life cycle and were of normal size. Eggs taken from the resulting flies hatched normally.

When 20 newly emerged females were fed on 1 mg./ml. of the potassium salt of gibberellic acid in skimmed milk, ovarian growth at the end of 4 days was the same as that of the control.

Each of 10 newly emerged female flies was treated topically on the dorsal thorax with 1.72 μg. of the test material, and then fed on skimmed milk for 3 days. Ovarian growth and survival were normal.

Dosages of 43.0, 4.3, and 0.43 μg. per fly in sterile distilled water were injected with a sterile syringe into the ventral abdomen, 20 newly emerged female flies being treated with each dosage. After 4 days there were no mortalities, and the ovaries showed normal growth in all flies. Evidently gibberellic acid at these dosages has no toxic or growth inhibiting effect. This lack of toxicity, if it may be applied to other insects, could be of importance to those applying gibberellic acid to plants, since the danger of affecting insect pollinators and predators would be small.

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[2] The sample used in this study was supplied by Merck & Company.

Relation of Structure of Diphenylmethane Derivatives to Toxicity to *Tribolium confusum* Duv.[1]

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ABSTRACT

The toxicities to the confused flour beetle, *Tribolium confusum* Duv., of 44 compounds related to diphenylmethane were compared. Two toxic mechanisms are suggested by time-mortality relationships as compared with dosage-mortality relationships. A DDT-type toxic mechanism appears to hold for the aliphatic substituted compound, while a more rapid diphenylmethane-type toxic mechanism is indicated for those with less aliphatic substitution. Replacement of benzene ring systems by other aromatic systems did not appreciably alter toxicity relationships.

In a previous communication, Gunther *et al.* (1954) introduced and discussed the "topographical" representation of one phase of a mechanism of insecticide-enzyme interaction. Briefly, the actions of contact insecticides upon enzyme systems to produce lethal effects involve phases of penetration, interaction between the insecticide molecule and the enzyme of interest, and ultimately the biochemical consequences of this interaction, which in favorable instances result in the death of the insect. Gunther *et al.* (1954) have been interested in the conditions or parameters qualifying the insecticide-enzyme interaction, and have found that with DDT-type compounds and mosquito larvae, complementary convolutions on the molecular scale (Pauling *et al.* 1942, 1945) are apparently essential. This suggests that a foreign biologically active molecule slips, in part at least, into a cav-

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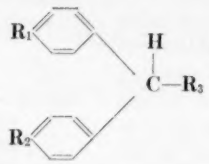
Table 1.—Diphenylmethane-type compounds, their melting or boiling points in °C., and their methods of synthesis (in parentheses).



R_3	$R_1=H$ $R_2=H$	$R_1=H$ $R_2=Cl$	$R_1=H$ $R_2=CH_3$	$R_1=Cl$ $R_2=Cl$	$R_1=Cl$ $R_2=CH_3$	$R_1=CH_3$ $R_2=CH_3$
CCl_3	64.8–5.8 (a)	73–4 (b)	66.6–7.4 (b)	107.5–8.5 (c)	87–8 (b)	86.5–7.0 (b) (a)
$CHCl_2$	77–8 (d)	119.5–20.1 (j)	94.0–4.4 (k)	108–9 (c)	97.4–8.4 (l)	77.8 (d)
CH_2Cl	37.5–8.0 (m)	40.3–1.0 (n)	140/2 mm. (o)	53.8–4.4 (e)	31.0–1.5 (p)	59.0–9.8 (q)
CH_3	117–8/2 mm. (f)	122/3 mm. (r)	111/3.5 mm. (s)	54–5 (g)	131/3 mm. (t)	137–8/4 mm. (h)
CH_2CH_3	107–8/5 mm. (i)	139/1.5 mm. (u)	129–30/3 mm. (v)	163–5/1 mm. (w)	134–5/3.5 mm. (x)	34–5/5 mm. (y)
H	25.8–6.0 78–9/1 mm. (z)	9–10 115–6/2 mm. (aa)	6–7 92–6/0.7 mm. (ab)	54–5 (ac)	31.5–2.5 (ad)	27–8 (ae)

(a) Harris & Frankforter, *Jour. Amer. Chem. Soc.* **48,** 3144 (1926).
(b) Chattaway & Muir, *J. Chem. Soc.* **1934,** 701.
(c) Mosher *et al.*, *Indust. and Engineer. Chem.* **38,** 916 (1946).
(d) Buttenberg, *Ann.*, **279,** 334 (1893).
(e) Cristol *et al.*, *Jour. Amer. Chem. Soc.* **74,** 3333 (1952).
(f) Klages & Heilman, *Ber.*, **37,** 1447 (1904).
(g) Grummett, Buck, and Becker, *Jour. Amer. Chem. Soc.*, **67,** 2265 (1945).
(h) Reichart & Nieuwland, *Org. Syn.*, Coll. Vol. 1, 229.
(i) Klages, *Ber.*, **35,** 2648 (1902).
(j) C: calcd. 58.87%, found 58.87%; H: calcd. 3.88%, found 4.00%.
(k) C: calcd. 67.94%, found 67.98%; H: calcd. 5.32%, found 5.45%.
(l) C: calcd. 60.13%, found 59.99%; H: calcd. 4.37%, found 4.49%.
(m) C: calcd. 77.59%, found 77.99%; H: calcd. 6.05%, found 6.15%.
(n) C: calcd. 66.95%, found 67.28%; H: calcd. 4.82%, found 5.28%.
(o) C: calcd. 78.08%, found 78.59%; H: calcd. 6.55%, found 6.90%.
(p) C: calcd. 67.94%; found 68.66%; H: calcd. 5.32%, found 5.11%.
(q) C: calcd. 78.51%, found 78.55%; H: calcd. 7.00%, found 7.11%.
(r) C: calcd. 77.59%, found 77.99%; H: calcd. 6.05%, found 6.22%.
(s) C. calcd. 91.78%, found 91.82%; H: calcd. 8.22%, found 8.17%.
(t) C: calcd. 78.08%, found 78.47%; H: calcd. 6.55%, found 6.52%.
(u) C: calcd. 78.08%, found 78.23%; H: calcd. 6.55%, found 6.52%.
(v) C: calcd. 91.37%, found 91.38%; H: calcd. 8.63%, found 8.85%.
(w) C: calcd. 67.94%, found 67.97%; H: calcd. 5.32%, found 5.71%.
(x) C: calcd. 78.51%, found 78.61%; H: calcd. 7.00%, found 6.76%.
(y) C: calcd. 91.01%, found 91.38%; H: calcd. 8.99%, found 8.74%.
(z) See "Diphenylmethane" in this section.
(aa) Tschitschebabin & Schlessler, *Chem. Zentr.*, 1926, *I*, 919.
(ab) v. Auwers and Frühling, *Ann.*, **422,** 224 (1921).
(ac) Jaeger, *Rec. trav. chim.*, **25,** 390 (1906).
(ad) New compound, proof of structure will be published elsewhere.
(ae) Lavaux, *Compt. Rend.*, **152,** 1401 (1910).

ity in the protein moiety of the enzyme and is held there tightly to inhibit the normal subsequent chemistry of the enzyme system. If the three-dimensional fit into the cavity is poor, or if the retention of the enclosed portion is poor, the degree of inhibition is lessened. This topographical representation therefore involves both concepts of "fit" (complementariness) and "hold" (polarizability) as separate but necessary conditions for this type of biological activity.

As discussed by Gunther *et al.* (1954), this concept is based upon the approximate interchangeability of methyl groups and chlorine atoms in biological systems, providing the "hold" condition is also satisfied. With DDT-type compounds wherein the chlorine atoms are variously replaced by methyl groups, DDT-type toxicity to the larvae of the southern house mosquito (*Culex quinquefasciatus* Say), is correlatable with the van der Waal's attractive forces of the substituent groups summed over the entire insecticidal molecule.

Although this correlation exists when mosquito larvae are used as the test organism, and appears to exist with the house fly (*Musca domestica* L.), and the greenhouse thrips (*Heliothrips haemorrhoidalis* Bouché), the correlation of DDT-type toxicity with van der Waal's attractive forces is not so apparent when testing with the confused flour beetle (*Tribolium confusum* Duv.). With this test organism, toxicity appeared to be conversely proportional to the length and degree of substitution of the aliphatic portion of the molecule, except DDT. It was therefore of interest to expand the series of compounds to be tested to the ultimate homomorph of this series, diphenylmethane, and its methyl and chlorine substitution products. The present data are therefore concerned with the toxic effects of these compounds on the confused flour beetle.

MATERIALS AND METHODS.—*Compounds.*—In table 1 are listed the diphenylmethane-type compounds, their melting or boiling points, and their methods of synthesis. (Details of the synthesis of new compounds will be reported elsewhere.) With some insecticides, benzene, thiophene, and other rings are frequently interchangeable without destroying the toxicity of the isosterically related compounds (Blinn *et al.* 1954, Metcalf & Gunther 1947); in these applications the rings seem to act as spacer groups with polarizable characteristics (Blinn *et al.* 1954). Therefore, the compounds listed in table 2 were also prepared and evaluated against the confused flour beetle.

Diphenylmethane.—A 5-gallon sample of diphenylmethane (n_D^{29} 1.5732) was used as the reservoir of material of constant composition for all tests. Its ultraviolet and infrared absorption characteristics indicated traces of aliphatic ketone or aldehyde, of benzophenone, and prob-

Table 2.—Miscellaneous compounds related to diphenylmethane.

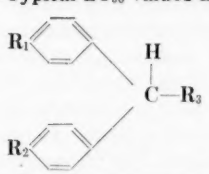
COMPOUND	M.p., °C.	REFERENCE
2-Thienylphenylmethane	17.5–8.5[a]	aa
Di-2-thienylmethane	43.6–5.3	ab
Cyclohexylphenylmethane	88.5/2.8 mm.[b]	ac
Benzophenone	Several	ad, ae
Benzhydrol	68–9	ad
Benzhydryl chloride	109–10/1.5 mm.[b]	ad
4,4'-Dichlorobenzhydryl chloride	60–1	ad
Diphenyl	69–71	ae

[a] Literature (aa) reports b.p. 129–35° C./10 mm. with no mention of crystalline compound.
[b] Boiling point.
(aa) Steinkopf & Schubart, *Ann.*, **424**, 1–23 (1921).
(ab) Blicke & Burckhalter, *Jour. Amer. Chem. Soc.* **64**, 478 (1942).
(ac) Sidorova & Tsakuranik, *Jour. Gen. Chem.*, **10**, 2073 (1940); through *Chem. Abstracts*, **35**, 980 (1941).
(ad) Available commercially in reagent grade.
(ae) Hodgman, Editor in Chief. "Handbook of Chemistry and Physics," 24th Ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1952.

ably of some diphenyl carbinol. Accordingly, the material was fractionated through a 2-foot Wheeler Center-Rod column to afford a colorless major cut (b.p. 78-9°/1 mm., n_D^{30} 1.5729). After repeated recrystallizations from low-boiling petroleum ether this material had m.p. 25.8 to 26.0°, n_D^{30} 1.5728. In 2×10^{-3} molar 2,2,4-trimethylpentane solution the recrystallized compound had the following constant A_s values: 380 at 269.5mμ, 448 at 262.5mμ, 436 at 260.0 mμ, 370 at 256.0 mμ, 373 at 254.0 mμ, and 271 at 249.5 mμ. Infrared scanning of the recrystallized compound indicated that no impurities, including compounds with oxygen functions, were detectable with 10% carbon tetrachloride or carbon disulphide solutions in 0.1-mm. and 1.0-mm. cells and with sodium chloride optics. After storage at room temperature for 1 year a new infrared spectrogram still demonstrated the absence of ketone and carbonyl functions in the recrystallized cut.

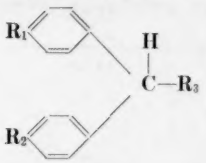
Bioassay.—Whole wheat flour was ground with varying weight-percentages of a given compound for 15 minutes

Table 3.—Typical LT_{50} values in hours.



R_3	Conc. %	R_1=H R_2=H	R_1=H R_2=Cl	R_1=H R_2=CH_3	R_1=Cl R_2=Cl	R_1=Cl R_2=CH_3	R_1=CH_3 R_2=CH_3
CCl_3	5	>360	168	<360	95	168	>360
	1	>360	>360	<360	110	258	>360
	0.2	>360	>360	>360	135	>360	>360
$CHCl_2$	5	>360	360	275	72	145	290
	1	>360	>360	>360	200	220	>360
	0.2	>360	>360	>360	>360	>360	>360
CH_2Cl	5	360	360	>360	160	>360	>360
	1	>360	>360	>360	330	>360	>360
	0.2	>360	>360	>360	>360	>360	>360
CH_3	5	< 72	< 72	< 72	160	< 72	< 72
	1	130	>360	>360	290	< 72	>360
	0.2	>360	>360	>360	>360	>360	>360
CH_2CH_3	5	< 72	< 72	< 72	155	130	>360
	1	168	72	>360	140	320	>360
	0.2	>360	>360	>360	>360	>360	>360
H	5	< 72	< 72	< 72	115	< 72	106
	1	< 72	92	>360	97	< 72	>360
	0.2	>360	>360	>360	>360	>360	>360

Table 4.—Typical LD_{50} values in per cent concentration.



R_3	Hours	R_1=H R_2=H	R_1=H R_2=Cl	R_1=H R_2=CH_3	R_1=Cl R_2=Cl	R_1=Cl R_2=CH_3	R_1=CH_3 R_2=CH_3
CCl_3	360	>5	2.2	>5	<0.2	0.75	>5
	168	>5	5	>5	<0.2	5	>5
	72	>5	>5	>5	0.9	>5	>5
$CHCl_2$	360	>5	5	4.2	0.45	0.7	4.2
	168	>5	>5	>5	2.2	2.7	>5
	72	>5	>5	>5	5.0	>5	>5
CH_2Cl	360	5.0	5.0	>5	0.9	3.0	>5
	168	>5	>5	>5	4.6	>5	>5
	72	>5	>5	>5	>5	>5	>5
CH_3	360	0.9	1.0	3.7	0.8	0.45	3.7
	168	0.9	2.2	4.0	4.4	0.7	3.8
	72	3.7	3.7	4.1	>5	0.8	4.4
CH_2CH_3	360	0.9	0.9	3.7	0.7	0.8	>5
	168	1.0	0.95	4.2	0.8	4.4	>5
	72	1.3	1.0	4.5	>5	5	>5
H	360	0.8	0.8	3.2	0.4	0.8	3.9
	168	0.8	0.8	3.2	0.5	0.8	4.2
	72	0.75	3.1	3.4	>5	0.75	>5

in an automatic stainless steel mortar and pestle unit. Prior tests with flour and a crystalline dye were made to establish optimum grinding conditions affording a ground flour with uniformly distributed microcrystalline particles of dye. The concentration series included 0.2, 0.5, 1.0, and 5.0% concentrations by weight of the compounds in the flour. Immediately after being ground, each mixture was transferred to a half-pint brown glass bottle which was capped with a pressure cap with aluminum foil liner then stored under refrigeration; this procedure mini-

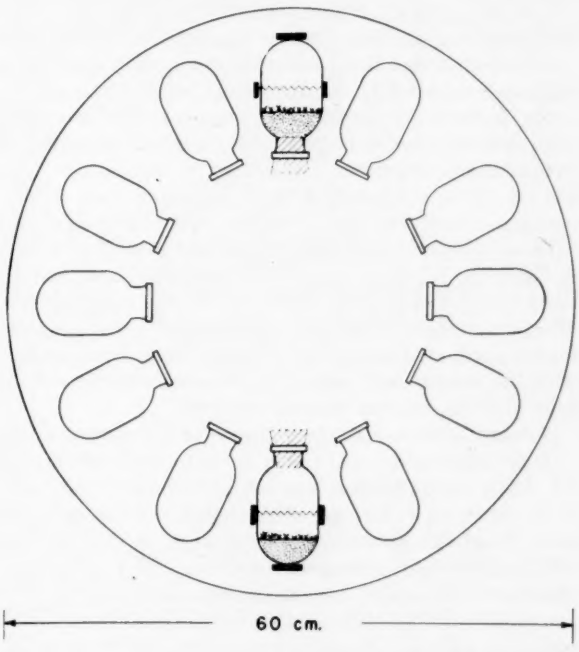


FIG. 1.—Diagram of mixing wheel used for comminuting the test compounds with flour, the wheel mounted directly on the drive shaft of a 30-to-1 gear reducer coupled to a 1725 r.p.m. motor.

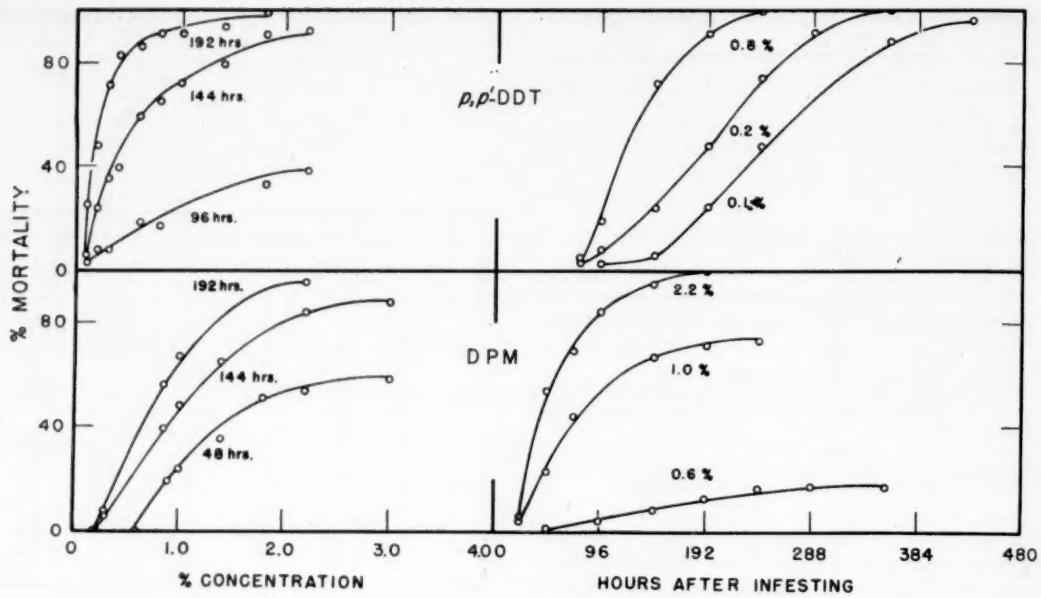


FIG. 2.—Typical time-mortality and dosage-mortality curves for unsubstituted diphenylmethane (DPM) and for *p,p'*-DDT used against the confused flour beetle.

mized possible losses of compound by volatilization during storage. Each bottle was shaken mechanically for 5 minutes before being opened.

The grinding and mixing procedure described above was developed after many tests. Accuracy and reliability of feeding and contact-action assay with the confused flour beetle are dependent upon a uniformly mixed product of very small particle size. If, for example, a test compound is repellent, the beetle will avoid eating the treated flour unless the chemical is so finely ground and so intimately mixed with the flour that it cannot be avoided.

Satisfactory grinding and mixing of crystalline compounds with larger quantities of flour were also achieved with a mixing wheel (see diagram fig. 1). The bottles were 200 ml. in capacity, and were of the heavy wall, round-bottom centrifuge type, capped with polyethylene-covered stoppers. Into each bottle was placed 20 grams of flour, the requisite amount of test compound, and 30 paint-free 7/8 inch metal "jacks" of the toy store variety. Thirty minutes on the mixing wheel at 58 r.p.m. afforded adequate mixing. Liquid compounds were handled with the mortar and pestle unit because they tended to cake with the flour on the mixing wheel.

Twenty adult beetles were placed in 5 grams of a test mixture contained in a 1-ounce salve tin with perforated lid. Each concentration was replicated four times, and four untreated controls were included in each series of tests. Mortality counts were made after 24, 48, 72, 120, 168, and 360 hours storage in the dark at 76° F. Moribund insects were counted as live insects. The insects were screened from the flour and counted, dead insects were removed, and the remaining live insects were returned to the flour and the tin for recounting after the next storage interval.

RESULTS.—Results were accumulated on the basis of both time-mortality and dosage-mortality relationships. With mortality counts of the beetles at 24, 48, 72, 120 168, and 360 hours and at 0.2, 0.5, 1.0, and 5.0% concentrations of compound in the flour, it was possible to plot the data to secure approximate LT_{50} and LD_{50} values for each compound. Typical curves for these values for unsubstituted diphenylmethane and for *p,p'*-DDT are shown in figure 2. The LT_{50} and LD_{50} values for the carefully purified diphenylmethanes and those listed in table 1 are collated in tables 3 and 4 and for the other carefully purified miscellaneous compounds in table 5. Each value is the average of four replicated tests.

DISCUSSION.—These data incorporate both LT_{50} and LD_{50} values to indicate possible differences in toxic mechanisms. For example, diphenylmethane kills very quickly at high concentrations but possesses little toxicity at lower concentrations by comparison, DDT kills much more slowly but possesses considerable toxicity at the lowest concentrations tested. It is interesting to note the difference in performance between diphenylmethane and 4,4'-dichlorodiphenylmethane. From the LT_{50} values in table 3, it is evident that diphenylmethane possesses a faster toxic action; from the LD_{50} values in table 4, however, 4,4'-dichlorodiphenylmethane possesses greater toxicity during the longer test periods.

It is also evident from the LT_{50} data in table 3 that fast toxic action of the diphenylmethane type is inversely proportional to the chain length and degree of substitution of the aliphatic portion of the molecule; toxicity appears to reside with chlorine or hydrogen substitution of the aromatic portion of the molecule, whereas methyl substitution lessens toxicity.

From the LD_{50} data in table 4, it appears that there is limited correlation of the DDT-type toxicity of the diphenylethanes and the van der Waal's attractive forces previously discussed (Gunther *et al.* 1954). However, the diphenylmethane-type toxicity which resides in the

Table 5.—Typical LT_{50} values and LD_{50} values for miscellaneous compounds.

Compound	LT_{50} Concentration Per Cent	LT_{50} Hours	LD_{50} Hours	LD_{50} Concentration Per Cent
2-Thienylphenylmethane	5	< 72	360	0.8
	1	80	168	0.8
	0.2	>360	72	1.1
Di-2-thienylmethane	5	< 72	360	0.7
	1	< 72	168	0.8
	0.2	>360	72	0.9
Cyclohexylphenylmethane	5	< 72	360	0.9
	1	94	168	0.9
	0.2	>360	72	2.3
Diphenyl	5	< 72	360	0.2
	1	80	168	0.7
	0.2	>360	72	0.8
4,4'-Dichlorodiphenyl	5	>360	360	>5
	1	>360	168	>5
	0.2	>360	72	>5
4,4'-Ditolyl	5	>360	360	>5
	1	>360	168	>5
	0.2	>360	72	>5
Benzhydryl chloride	5	100	360	—
	1	>360	168	—
	0.2	>360	72	—
Benzhydryl bromide	5	360	360	—
	1	>360	168	—
	0.2	>360	72	—
4,4-Dichlorobenzhydryl chloride	5	240	360	—
	1	>360	168	—
	0.2	>360	72	—
Benzhydrol	5	>360	360	>5
	1	>360	168	>5
	0.2	>360	72	>5
Benzophenone	5	>360	360	>5
	1	>360	168	>5
	0.2	>360	72	>5

aliphatic unsubstituted diphenylethanes and propanes contraindicates their inclusion in this correlation.

The data presented in table 5 suggest that the diphenylmethane-type toxicity resides in compounds with an aliphatic substituted aromatic system. Further work is contemplated to explore this indicated toxicity trend.

Agreement among replicates in a given series of tests was excellent, but variations among the averages of series repeated at different times were considerable and amounted to as much as 600% in the LT_{50} values and 1000% in the LD_{50} values. Typical LD_{50} and LT_{50} values

Table 6.—Variations among the average of replicated[a] series of tests run at different times with *p,p'*-DDT and DPM (diphenylmethane).[b]

LT_{50} DDT	LT_{50} DPM	LD_{50} DDT	LD_{50} DPM
150 hr. at 5%	80 hr. at 5%	0.4% at 72 hr.	1.8% at 48 hr.
80 hr. at 5%	12 hr. at 5%	1.0% at 72 hr.	1.4% at 60 hr.
56 hr. at 1%	20 hr. at 1%	0.2% at 120 hr.	5.0% at 60 hr.
72 hr. at 1%	40 hr. at 1%	0.6% at 120 hr.	0.4% at 72 hr.
120 hr. at 1%	100 hr. at 1%	0.8% at 120 hr.	1.0% at 72 hr.
168 hr. at 0.2%	60 hr. at 1%	0.2% at 192 hr.	0.8% at 100 hr.
120 hr. at 0.2%	72 hr. at 0.2%	0.1% at 240 hr.	1.0% at 100 hr.

[a] Four times, with appropriate controls.
[b] The LT_{50} values and the LD_{50} values are from separate experiments.

accrued in separate tests for DDT and diphenylmethane are presented in table 6 to illustrate this variability.

The reasons for these large variations are not clear, but the possibility of the compound being highly repellent to the beetles was evaluated in the apparatus diagrammed in figure 3. Eight symmetrically placed feeding chambers were filled at random in repeated tests with whole wheat flour and with whole wheat flour containing 5% toxicant. One hundred beetles were placed in the central chamber. The entire assembly was placed at room temperature in the dark, and the number of beetles in each feeding chamber was determined at 24-hour intervals. Significant differences between effects of the treated and the untreated whole wheat flour did not develop; the beetles migrated freely from chamber to chamber as denoted by fluctuating counts in each.

CONCLUSIONS.—The toxicities to the confused flour beetle of 44 compounds related to diphenylmethane were compared. Two toxic mechanisms are suggested by time-mortality relationships as compared with dosage-mortality relationships. A DDT-type toxic mechanism appears to hold for the aliphatic substituted compound, while a more rapid diphenylmethane-type toxic mechanism is indicated for those with less aliphatic substitution. Replacement of benzene ring systems by other aromatic systems did not appreciably alter toxicity relationships.

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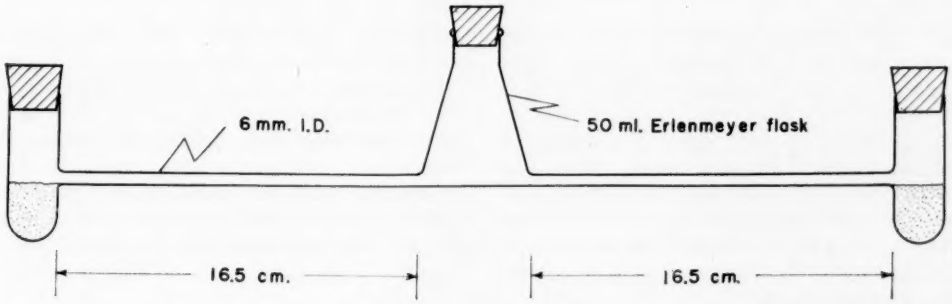

FIG. 3.—Cross-sectional diagram of a glass device to evaluate the repellent effect of diphenylmethane.

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Biological Control Agents and Toxicant Producing Plants Introduced into Indonesia[1]

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ABSTRACT

The following biological control agents and toxicant producing plants have been imported into Indonesia as part of the ICA program: *Melcha ornatipennis* Cam. from India for control of *Scirpophaga* rice and cane borers; *Schizonotus latus* (Wlkr.) from United States for control of *Phaedonia inclusa* (Stål) soybean beetle; *Lamprophorus tenebrosus* (Walker) from Ceylon for *Achatina fulica* Fer., African snail; *Orius insidiosus* (Say) from the United States as a general predator, especially of earworm eggs, aphids and thrips on corn; *Apantales chilonis* Mun, *Chelonus munakatae* Mun., *Microgaster russata* Hal., *Cremastus chinensis* (Vier.), *Casinaria* sp. from Japan for control of rice borers. Only *S. latus*, *O. insidiosus*, *A. chilonis*, and *C. munakatae* were released in the field. No evidence of establishment has been noted. Further efforts to establish *M. ornatipennis* and *S. latus* are recommended. Because of its susceptibility to predation by village chickens, *L. tenebrosus* is not considered to have good prospect of being of material value in controlling *A. fulica*.

Perezia pyraustae Pail. has been imported and applied in the field to rice and cane borer eggs and larvae; *Bacillus thuringiensis* Berl. has been used on field populations of *Plutella maculipennis* (Curt.); from Ceylon a polyhedrosis virus of *Prodenia* sp. has been recently received. Sabadilla, *Schoenocaulon officinale* Schlecht. and Champ., and red squill, *Urginea maritima* Baker were imported and are now multiplying in various field areas.

Teleonemia scrupulosa (Stål) was transferred from Java to Timor for the control of lantana and is now established there.

Parasites, predators and toxicant producing plants were introduced into Indonesia as part of the program of the U. S. International Cooperation Administration (ICA) in economic entomology. Unfortunately most of the biological control agents introduced were not released in large numbers over a long period of time because an adequate laboratory breeding program in conjunction with releases could not be established. In spite of an almost complete absence of cooperating entomologically trained personnel, efforts at importing these biological control agents were made because of the attractiveness of a self-supporting, self-spreading program to help the untrained, low income farmers.

The introductions cited here were all received during the period 1954 to 1956. Further work in this line has been stopped because of the difficulties of making arrangements for shipments and the establishment of a chemical control program which is considered within the range of the local farmers.

Melcha ornatipennis Cam., a common parasite of the larva of the sugarcane borer (*Scirpophaga nivella* (F.)) and occasionally rice borers in India was imported as a parasite of the cane and rice borers (*S. nivella* and *S. innotata* (Wlk.)) in Indonesia. Out of a trial shipment of less than 40 pupae, about 10 females emerged. Adequate financial arrangements for collection and shipment of sufficient numbers for field release and possible laboratory breeding could not be made. The trial shipment was sent by Sri B. D. Gupta, Sugar Cane Entomologist, Sugar Research Station, Lucknow, India.

Schizonotus latus (Wlkr.), an important parasite of the willow leaf bettle, *Plagiodera versicolora* (Laich.), in the United States and Europe, was imported as a potential parasite of the soybean beetle, *Phaedonia inclusa* (Stål). The first shipment of these parasites was prepared and shipped by Dr. John C. Schread, Entomology Department, Connecticut Agricultural Experiment Station, New Haven, Conn. All individuals were dead on arrival, probably because the air shipment took nearly a month in transit.

A second collection of *S. latus* made by Dr. Philip Dowden, Northeastern Forest Experiment Station, U. S. Dept. of Agriculture, New Haven, Conn., was shipped through the cooperation of Moorestown, N. J. Laboratory, Entomology Research Division, U. S. Dept. of Agriculture. This collection was sent in two shipments about 3 weeks apart during September and October 1956; some individuals in each shipment arrived alive even though the air shipments took over 2 weeks in transit.

All of the *S. latus* were shipped as adults in order to eliminate any chance of hyperparasites arriving in Indonesia, a precaution required because of the lack of adequate quarantine facilities. None of the females ever attempted oviposition in the laboratory even though they were exposed to two chrysomelinae hosts, *Phaedonia inclusa* and *Phyllocharis undulata* (L.). It is believed that the lack of attempts at oviposition can be associated with the fact that these adults were in hibernation diapause at the time of shipment from the United States. About 100

[1] Accepted for publication January 9, 1958.

adults were liberated in a soybean field at Kediri, East Java, and about 50 in Bogor, West Java, in a field of *Desmodium* sp., a plant also attacked by *Phaedonia indusa*.

Lamprophorus tenebrosus (Walker), a Lampyrid predator of *Achatina fulica* Fer., was shipped from Ceylon by Dr. Henry A. Bess of the Entomology Department, Agricultural Experiment Station, Honolulu, Hawaii. About 75 individual larvae of varying ages were received alive. These were kept in the laboratory and for a while did well feeding on 1-month-old *Achatina*. The laboratory population all died within 3 months of arrival in Bogor; only one generation a year is reported. The Bogor Laboratory population probably was killed by mites which came in with the snails. No individuals of this shipment were liberated in the field. It is now believed that this predator would not have a good chance of establishment and economic value in this area because of the apparent extreme vulnerability of the larvae to the ubiquitous chickens. *Achatina* is primarily a pest in village and urban areas in contrast to fields beyond the villages; the chickens are numerous, free roaming predators in all inhabited areas. Unfortunately, they hardly ever eat the snail.

Orius insidiosus (Say) adults were collected and shipped by D. W. Jones of the Moorestown, New Jersey Laboratory, Entomological Research Division, U. S. Dept. of Agriculture. About 1000 adults were shipped and almost 100% were alive on arrival 10 days later. Most of these were liberated in corn fields in Bogor and Bandung, West Java. This predator was hoped to be an aid in the control of the corn earworm and other Phalaenidae which are important pests on corn. In addition economic populations of aphids and thrips on corn are also common.

About 5000 hibernating larvae of *Chilo suppressalis* (Wlkr.) from Japan were shipped by Dr. Kawada, Plant Pathology and Entomology Section, National Institute of Agricultural Sciences, Tokyo, Japan. Out of this shipment a few thousand adults of *Apanteles chilonis* Mun. were reared; these were liberated in infested rice fields in Bogor and Krawang, West Java. Relatively few individuals of *Microgaster russata* Hal., *Chelonus munakatae* Mun., *Cremastus chinensis* (Vier.), and *Casinaria* sp. also emerged. Some of the *Chelonus munakatae* were liberated in Bogor and Krawang also. In the laboratory, about one-third of the hibernating *Chilo* larvae developed a fungus infection which was thought to be *Beauveria bassiana* Bals. The parasites which emerged from this shipment were identified by Luella M. Walkley and C. F. W. Muesebeck (1956).

Perezia pyraustae Pail., a protozoan disease of the European corn borer larva, was sent by Dr. Henry Townes, then ICA Entomologist in the Philippines. Hundreds of egg clusters and larvae of both cane and rice borers were inoculated in the field with this material. Work was done in the Bogor and Tjirebon areas of West Java.

A culture of *Bacillus thuringiensis* Berl. was given to R. Soepartono Siswopranoto, an ICA plant pathology participant, while he was in the United States for training. This culture was supplied by Dr. I. Tanada, Entomology Department, Agricultural Experiment Station, Honolulu, Hawaii. The culture was brought to the Plant Pest and Disease Institute, Bogor, where Mr. Soepartono is in charge. Mr. Ida Njoman Oka (1957) undertook laboratory study and culture of the organism and recently has tried it in the field in various areas of Java on *Plutella maculipennis* (Curt.). Encouraging success has resulted in the field tests already made.

A polyhedrosis virus collected from *Prodenia* sp. in Ceylon has been sent to Bogor by Dr. Paul Surany, Insect Pathologist, Oryctes Control Project, Pacific Science Board, National Research Council. This virus was received in 1957 and has not yet been tried on the local species of *Prodenia*.

No intensive effort has been made to check the establishment in the field of the liberated parasites or predators. Casual field observations in the areas of liberation have not indicated any establishment. If, when adequate facilities for laboratory breeding in Indonesia are available, a detailed field survey indicates that *Schizonotus latus* has not become established, it is the opinion of this technician that further efforts to establish both *Melcha ornatipennis* and *Schizonotus latus* would be worth while.

Seeds of sabadilla, *Schoenocaulon officinale* Schlecht. and Champ., collected in Colombia, were received through the cooperation of Mr. Howard Hyland, Plant Introduction Section, Horticultural Crops Research Branch, U. S. Dept. of Agriculture, Beltsville, Maryland. The seeds were planted in research station gardens in Bogor, West Java; near Malang, East Java; and in an upland garden in Central Sumatra.

About 25 bulbs of red squill, *Urginea maritima* Baker, were shipped by Mr. Hyland from a collection made in California. These bulbs were also planted in various research station gardens. Some were held in Bogor, others were planted near Surabaya, East Java; some in Makassar, Sulawesi; and some in Ende, Flores.

The red squill and sabadilla material is growing and slowly multiplying in numbers. It is hoped that economic amounts will become available while the products of these plants are still important in pest control work.

The writer, at the request of a committee of leaders of the Indonesian Ministry of Agriculture, transferred to the island of Timor about 4000 adults of *Teleonemia scrupulosa* (Stål) collected in Bogor, Java. Liberation of these insects was made in December 1954 in about five places along the 100-mile road from Kupang to Soie. Within a year establishment in many areas was evident (Ashadi 1957).

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A Study of Residues of Some Commonly Used Insecticides on Alfalfa[1]

J. R. Dogger[2] and T. G. Bowery[3,4]

Abstract

Alfalfa treated with dieldrin, heptachlor and malathion at practical rates was sampled at intervals and analyzed for insecticide residues. Heptachlor and malathion residues on green forage were below tolerance levels within 10 days. Residues from granulated heptachlor applied at 3 and 5 pounds per acre persisted for 20 to 30 days. Dieldrin spray residues of over 0.1 p.p.m. persisted after 30 days. Residues from dieldrin and malathion dusts were less persistent than those from sprays.

The spread of the alfalfa weevil, *Hypera postica* (Gyll.), and the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), into North Carolina since 1955 has greatly increased the interest in the use of insecticides on alfalfa. Materials which might be used to control these insects include heptachlor and dieldrin for the control of the alfalfa weevil and malathion for aphid control. Heptachlor and dieldrin have further use, at higher rates, in the control of soil insects.

Tolerances for residues of these materials, established under the Miller Pesticide Residue Amendment to the Federal Food, Drug and Cosmetic Act, are as follows: heptachlor 0.1 p.p.m., dieldrin zero and malathion 8 p.p.m.

The present study was made to determine what insecticide residues might be found at various intervals after the application of practical dosages of these insecticides to alfalfa in the field.

Procedure.—Experimental alfalfa plots at the Central Experiment Station at Raleigh, N. C., were treated on a given date and sampled at intervals thereafter. The treatments were:

A. Dieldrin spray at 0.25 lb. technical per acre,
B. Dieldrin dust at 0.25 lb. technical per acre,
C. Heptachlor granules at 3 lbs. technical per acre,
D. Heptachlor granules at 5 lbs. technical per acre,
E. Heptachlor spray at 0.25 lb. technical per acre,
F. Heptachlor dust at 0.42 lb. technical per acre,
G. Malathion spray at 1 lb. technical per acre,
H. Malathion dust at 1.17 lbs. technical per acre, and
I. Untreated check.

Each treatment was applied to three plots of approximately 0.02 acre in area, so aligned that the plots treated similarly were adjacent and the plots treated with materials known or expected to be more residual were located at one end of the experimental area while the check plots were placed at the opposite end. Like plots thus lay end-to-end in the field and were treated by moving back and forth crossing the combined length of the plots as each "swath" was treated. The objective of this design was to prevent or minimize contamination during treatment or while sampling.

All plots were treated on June 13, 1956, at which time the second growth of alfalfa had reached a maximum height of 10 inches. The sprays were applied as emulsions with a Boyette self-propelled sprayer at the rate of 8.4 gallons of total liquid per acre. Dusts were applied with a rotary hand duster using 1.5% dieldrin, 2.5% heptachlor and 4% malathion. The granulated heptachlor was applied as a 2.5% formulation using a Gandy distributor.

Sampling was accomplished by cutting a 3-foot swath with a Tornado sicklebar type mower across the width of a plot, gathering the fresh-cut alfalfa and taking the desired amount of material from the aggregate. For sampling purposes each plot was divided into 13 three-foot swaths. At each sampling date one of these swaths was picked at random for cutting from each plot of the nine treatments. If more than one set of plots was to be sampled on the same date, work progressed from the check plots toward the plots treated with the more residual materials to minimize contamination by the mower, workers and equipment used in collecting the forage. Care was taken to avoid moving any alfalfa from one plot to another.

Forage samples were placed in paper bags, marked, and put into cold storage at 35° F. Bags from the three like plots were placed together in a larger paper sack to avoid cross-contamination through possible contact with differently treated samples. Samples were taken from all plots 1, 5, 10, 15, 20, and 30 days after treatment. Additional samples were taken in the heptachlor-treated and check plots on the third day after treatment. Records were kept of temperature and rainfall in the area during the period of study.

Samples from plots treated with heptachlor granules and from untreated check plots were sent to the Velsicol Corporation, Chicago, Illinois, for analysis. A complete series of field samples from all plots were delivered to the Pesticide Residue Laboratory, Chemistry Department, North Carolina State College, for subsequent processing and analysis.

The material from each of the three field plots was pooled, passed through a Haddon Organic Shredder, hand mixed and subsampled to give duplicate 500-gram ("as received basis") samples. The samples were then tumbled -extracted with Na-distilled n-hexane (dieldrin and malathion) and special colorimetric pentane (heptachlor) at a 5:1 (ml./gm.) ratio at approximately 50 r.p.m. for 60 minutes. The extracts were then filtered, and concentrated in Danish-Kuderma concentrators fitted with water-cooled Friederichs condenser heads. The concentrated extracts were transferred to 100-ml. volumetric flasks, made up to volume and stored at −20°C. until analyzed.

At the time of analysis, the volumetric flasks were re-

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[4] The authors wish to acknowledge the cooperation of the Shell Chemical Corporation, the American Cyanamid Company, and the Velsicol Corporation.

moved from cold storage and allowed to warm up to 25° C. at which time analytical samples of the concentrate equivalent to 100 and 300 grams of alfalfa were withdrawn and transferred to 25×200 mm. test tubes.

The 300-gram samples were used for malathion analysis utilizing the method of Norris *et al.* (1954) as modified by Conroy (1956) without any cleanup procedure.

In the heptachlor and dieldrin samples it was necessary to employ a dewaxing procedure prior to further cleanup and subsequent analysis due to the abundance of plant wax present in the extracts which would seriously intertere in the subsequent analysis.

This de-waxing was carried out by placing the test tubes holding the analytical samples in a 45° C. water bath under a filtered air manifold and evaporating to near dryness. The samples were then taken up in a minimum of hot acetone. The tubes and a supply of wash acetone were then chilled in a dry ice-acetone bath at −75° C. for 1 hour. The cold acetone isolubles precipitated out while the heptachlor and dieldrin remained soluble, and the insolubles were filtered off using medium porosity sintered glass Buchner funnels. The samples were then placed in a 45° C. water bath under a filtered air manifold and evaporated to near dryness. Hexane was added to the dieldrin samples and pentane to the heptachlor samples, and the evaporation procedure repeated twice to remove the last traces of acetone.

After de-waxing, the heptachlor samples were analyzed by the method set forth by Ordas *et al.* (1956) and the dieldrin samples were analyzed by the colorimetric procedure of O'Donnell *et al.* (1955).

RESULTS AND DISCUSSION.—The amounts of heptachlor residue found by chemical analysis on alfalfa at various intervals after treating are shown in table 1. The greater part of the heptachlor residue disappeared after 3 days at the low rates of application used on spraying and dusting and after 5 days at the higher rates used with the granules. With both spray and dust, the residue level was less than 0.1 p.p.m. 10 days after application. However, with the granules, the residues had not diminished to the tolerance level until 20 to 30 days after the field had been treated.

Data on dieldrin residues from alfalfa treated with spray and dust formulations are also presented in table 1. When dieldrin was applied as a dust the residue persisted in detectable amounts until the 20th day after the alfalfa had been treated. In the case of application as a spray a residue of over 0.1 p.p.m. persisted on the 30th day following treatment.

Data on residue present on the alfalfa at various intervals after treatment with malathion are also presented in table 1. One day following the application of malathion as a dust the residue present was found to be less than the tolerance of 8 p.p.m. It was not until the 10th day after application of the same material as a spray that residues had decreased below this level.

Information regarding the temperature and rainfall during the period in which the study was made is presented in table 2.

In interpreting the data presented, two things should be kept in mind. First, this study was conducted under conditions of high temperature. Residues might conceivably have been higher if the tests had been conducted under cooler conditions. Second, residues were determined on the basis of their proportion relative to the weight of green forage. Logically they would be higher if calculated on a dry weight basis. However, Poos *et al.* (1952) reported that in general less residue was present on dried forage samples than on undried samples treated with any one of four chlorinated hydrocarbon insecticides. Rai *et al.* (1957) showed that about 69% of malathion residues present on green alfalfa disappeared during dehydration processes.

The evidence obtained, indicating that malathion residue disappears in about 15 days, will permit the use of treated alfalfa after a very short waiting period.

The small amount of insecticide residue persisting on the alfalfa after the application of 0.25 to 0.42 pound of heptachlor indicates that a waiting period of 10 days or more before using alfalfa treated for weevil control might be safely recommended in North Carolina.

The more persistent heptachlor residues following the use of granulated formulations is believed to be associated largely with the rates of application, 3 and 5 pounds of technical material per acre. It is evident that the use of such formulations does not eliminate the prob-

Table 1.—Insecticide residues (p.p.m.) on green alfalfa forage at various intervals after treatment with several formulations at practical rates of application. Raleigh, N. C. 1956.

TREATMENT	DAYS AFTER APPLICATION						
	1	3	5	10	15	20	30
Heptachlor							
Spray emulsion at 0.25 lb./A.	0.72	0.22	0.08	0.07	0.07	0.07	0.07
Dust at 0.42 lb./A.	0.79	0.46	0.11	0.07	.07	.07	.07
Granules at 3 lbs./A. Chicago[a]	4.662	2.464	1.012	0.097	.169	.023	.078
Raleigh	3.30	2.67	1.92	0.97	.51	.19	.10
Granules at 5 lbs./A. Chicago	4.586	2.764	2.804	0.217	.072	.000	.091
Raleigh	4.81	3.60	1.93	1.27	.58	.15	.12
Dieldrin							
Dust at 0.25 lb./A.	3.42		2.55	1.12	0.87	0.05	0.05
Spray at 0.25 lb./A.	3.65		1.70	1.12	0.61	0.27	0.18
Malathion							
Spray at 1 lb./A.	46.60		8.08	0.76	0.41<0.20	<0.20	
Dust at 1.17 lbs./A.	2.00		0.82	0.25	0.21<0.20	<0.20	

[a] Analyses were run on duplicate subsamples of green forage taken in the field, Chicago analyses by the Velsicol Corporation; Raleigh analyses by the Pesticide Residue Laboratory, North Carolina State College.

Table 2.—Rainfall and mean maximum temperatures during the period of study of insecticide residues on alfalfa at Raleigh, N. C. 1956.

DATE	DAYS BETWEEN TREATMENT AND SAMPLING	RAINFALL (IN.)		MEAN MAXIMUM TEMPERATURE °F.	
		Since Last Sampling	Treatment to Sampling	Since Last Sampling	Treatment to Sampling
June 14	1		0.0	90	90
June 16	3	0.0	0.0	90	90
June 18	5	0.0	0.0	92	91
June 23	10	0.7	0.7	86	89
June 28	15	0.0	0.7	95	91
July 3	20	0.2	0.8	93	91
July 13	30	2.8	3.7	89	91

lem of insecticide residues, even though granules are less likely to adhere to plant surfaces than dust particles or spray droplets.

Residues of dieldrin used as a dust at 0.25 pound per acre were below 0.1 p.p.m. after 20 days. However, the residue still exceeded the zero tolerance set by Federal law. It appears that a more realistic approach could be used in setting tolerances of this kind, since the limits of sensitivity in the analytical chemistry techniques used prevent determining with certainty when "zero" is reached.

The residues found on alfalfa treated with either dieldrin or malathion indicate that these materials are much more persistent when applied as sprays than when applied at comparable rates as dusts.

CONCLUSIONS.—A study of insecticide residues on alfalfa following the application of various chemicals at practical rates revealed:

1. Dieldrin residues of over 0.1 p.p.m. may persist 30 days after application of this material at 0.25 pound per acre as a spray.
2. Detectable amounts of dieldrin may persist 15 days after application as a dust at 0.25 pound per acre.
3. The application of granulated formulations of heptachlor at 3 and 5 pounds per acre may result in residues above the tolerance level, 0.1 p.p.m., until 20 to 30 days after their use.
4. Heptachlor residues were less than 0.1 p.p.m. 10 days after the application of this material at rates of 0.25 to 0.42 pound per acre as a spray or dust.
5. Residues of malathion applied as a dust at approximately 1 pound per acre may diminish below the 8 p.p.m. tolerance level one day after application.
6. Malathion residue may diminish below the tolerance level by the 10th day after application of 1 pound per acre as a spray.

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Onion Thrips (*Thrips tabaci* Lind.) Infesting Cabbage[1]

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ABSTRACT

Infestations of thrips, principally *Thrips tabaci* Lind., but including small numbers of *Frankliniella fusca* (Hinds), *F. tritici* (Fitch), *F. exigua* Hood, and *F. tenuicornis* (Uzel) have seriously damaged maturing fresh market cabbage in production fields in eastern Iowa. On the basis of trapping records, the thrips apparently migrated from alfalfa and winter wheat where they had attained great numbers which were counted in periodic samplings. The thrips were not controlled by DDT nor, in fact, by other compounds after they had become established within the cabbage head.

A series of insecticides were compared in field plots in 1956 and 1957 through a method of visual rating of relative plant damage at harvest. Heptachlor was the most effective in preventing thrips damage. Following a weekly schedule of applications of heptachlor at one-half pound per acre the marketable crop was increased 30% above that of untreated check plots where losses ranged from 36 to 39% of the crop. The problem of thrips damage to cabbage, though not widely recognized is potentially serious.

The production of fresh-market cabbage along the Mississippi River in southeastern Iowa has been seriously imperiled by a russeting or bronzing of cabbage ready for harvest. Frequently entire plantings have been unfit for harvest or have been cut and shipped only to be refused at receiving docks following continuous thrips damage during transit. About 60 years ago Sirrine & Lowe (1894) and Quaintance (1898) described the minute silvery scars caused by the feeding of thrips upon the unexposed side of cabbage leaves when the heads began to form. As the heads matured they became discolored throughout from the coalescence of innumerable feeding scars. Except for these early reports there have been but few references to the problem in the literature.

The problem reported here has become severe following the continuous use of DDT since its introduction in the early 1940's for the control of the leaf-feeding larvae, *Plutella maculipennis* (Curt.), *Trichoplusia ni* (Hbn.), and *Pieris rapae* (L). DDT does not control the thrips at this time. Recently it had become the practice in this

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[2] Graduate Assistant and Associate Professor, respectively, Department of Zoology and Entomology. The writers gratefully acknowledge the determinations of thrips species by Miss Kellie O'Neill of the Insect Identification and Parasite Introduction Section of the Agricultural Research Service, U. S. Department of Agriculture.

area to apply DDT sprays to cabbage until head formation, then to shift to parathion sprays at 10- to 14-day intervals as the cabbage looper infestations increased. Under this practice the thrips infestations have flourished. The objectives of the research reported in this paper have been (1) to discover which species of thrips caused the damage, (2) to explore the possible sources of the infestations, and (3) to investigate the use of insecticides for the control of the damaging infestations.

METHODS.—Beginning just before heading was initiated, adult thrips for the determination of species were collected from cabbage plants. The collections were continued at intervals during the growing seasons of 1956 and 1957. In both years tanglefoot traps were erected to collect samples of the thrips moving into cabbage fields in order that periods of flight might be recorded. The traps consisted of insecticide drums inverted on stakes 6 feet in height. Index cards (5 inches wide and 8 inches long) surfaced with tangle-foot were secured to the drums to face the four cardinal directions at a height of 5 feet from the ground. Each week the trapped thrips were counted and new cards smeared with fresh tanglefoot were placed over the previous ones. In 1957 near the peak flight of thrips those captured on the traps were removed by dissolving the tanglefoot and filtering out the thrips by the method of Hightower & Martin (1956). In a search for possible sources of the infestations of cabbage, weeds and crop plants of the area were sampled for thrips. Four alfalfa and four winter wheat fields were sampled weekly from March 10 through September 3 by removing the plants and top half inch of soil from two areas (4 inches wide by 8 inches long) from one of four randomly selected quarter acres at four sites in each field (total sample area per field, 256 sq. in.). Thrips emerging from the samples dropped into jars of AGA killing solution (Bierne 1955) secured to the base of Berlese funnels while the samples were held in the funnels for 24 hours. The species of adult thrips were determined and a record was made of the number of adults and larvae taken.

Experiments were designed to evaluate selected insecticides and the timing of insecticide applications in the control of thrips. The plots were established in commercial plantings of the variety Racine. The evaluations of all the treatments involving insecticides were based upon the examination of individual cabbage heads in the field at harvest time. The intensity of thrips infestation and the extent of damage were observed when the head was cut from the stem, split into halves and the internal parts were exposed. A visual rating of relative infestation intensity and damage was assigned to each of four individual heads per plot in 1956 and to each of 20 heads per plot in 1957. Class one indicated the least infestation and damage; class five indicated the most severe infestation and damage. One-half of the ratings in each plot were made independently by two observers. The percentage of marketable heads in the 1957 experiments was derived from the frequency of occurrence of classes one through four. Those heads assigned class 5 were not marketable because of severe bronzing and the presence of numerous thrips. Data were statistically analyzed for sources of variance by applying the F test and the Tukey range test. A multiple range test outlined by Duncan (1955) was applied to aid in evaluating differences between treatment means in the comparison of weekly and biweekly schedules.

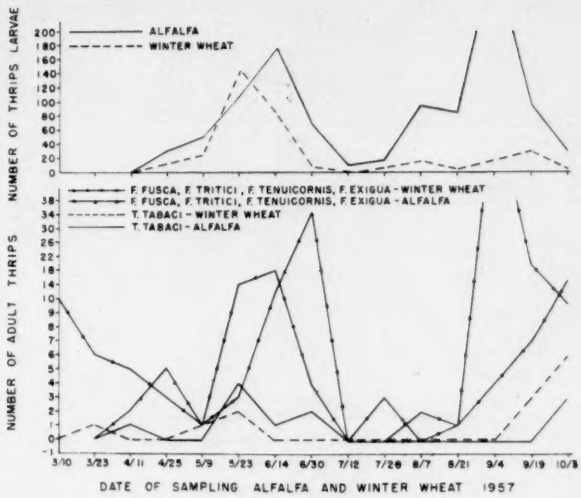
ALFALFA
WINTER WHEAT
NUMBER OF THRIPS LARVAE
F. FUSCA, F. TRITICI, F. TENUICORNIS, F. EXIGUA - WINTER WHEAT
F. FUSCA, F. TRITICI, F. TENUICORNIS, F. EXIGUA - ALFALFA
T. TABACI - WINTER WHEAT
T. TABACI - ALFALFA
NUMBER OF ADULT THRIPS
3/10 3/23 4/11 4/25 5/9 5/23 6/14 6/30 7/12 7/26 8/7 8/21 9/4 9/19 10/3
DATE OF SAMPLING ALFALFA AND WINTER WHEAT 1957

FIG. 1.—Mean number of thrips taken from four samples (64 sq. in. each) in periodic sampling of four alfalfa and four winter wheat fields. Muscatine County, Iowa. 1957.

RESULTS AND DISCUSSION.—Following the collection and identification of thrips from cabbage in the area, it was apparent that the onion thrips, *Thrips tabaci* Lind., was the principal species involved in the damage of cabbage. The tobacco thrips, *Frankliniella fusca* (Hinds), the flower trips, *F. tritici* (Fitch), as well as *F. exigua* Hood and *F. tenuicornis* (Uzel) were also taken in collections from cabbage. In the collection of thrips from native host plants and cultivated crops, the species named above appeared repeatedly from a wide range of plants. Since the onion thrips soon appeared to be the predominant species causing the damage to cabbage, the production fields of alfalfa and winter wheat were suspected sources of infestation. Periodic samplings proved that tremendous thrips populations including *T. tabaci* developed

Table 1.—Comparison of emulsifiable formulations of insecticides for control of thrips. August 10, 1956.

INSECTICIDE	POUNDS PER ACRE	MEAN VISUAL RATING OF THRIPS DAMAGE[a]
Heptachlor	0.50	1.3
Parathion	0.33	1.3
Dieldrin	0.25	1.5
Endrin	0.25	2.5
Thimet	1.00	2.7
Guthion	0.75	2.7
DDT	1.00	3.0
Demeton	0.50	3.5
Thiodan	0.50	3.7
Chlorthion	0.75	3.8
Methoxychlor	2.00	3.8
Dipterex[b]	1.00	4.2
Malathion	1.00	4.7
Untreated	—	5.0
$D_{.05}$ (Tukey range test)		2.2

[a] Range: 1, least damage; 5, greatest damage. Any two means paralleled by the same line are not significantly different.
[b] Soluble powder formulation.

Table 2.—Comparison of emulsifiable formulations of insecticides for control of thrips. July 23, 1957.

Insecticide	Pounds per Acre	Per Cent Marketable Heads	Mean Visual Rating of Thrips Damage[a]
Heptachlor	0.50	94	46.8
Parathion	0.30	85	48.6
Dieldrin	0.30	85	50.6
Thiodan	0.50	74	58.2
Phosdrin	0.30	69	59.6
Sevin[b]	1.00	74	59.6
Endrin	0.30	69	59.8
Guthion	0.75	69	64.4
Perthane	1.00	57	71.0
Untreated	—	64	73.6
$D_{.05}$ (Tukey range test)			20.8

[a] Range: 20, least damage; 100, greatest damage. Any two means paralleled by the same line are not significantly different.
[b] N-methyl-1-naphthyl carbamate.

Table 3.—Comparison of spray schedules in control of thrips, July 24, 1957.

	Pounds per Acre	Weekly or Biweekly	Per Cent Marketable Heads	Mean Visual Rating of Thrips Damage[a]
Heptachlor	0.50	Biweekly	77	45.7
Heptachlor	0.50	Weekly	93	46.0
Parathion	0.33	Weekly	84	49.0
Toxaphene	2.00	Biweekly	79	60.3
Parathion	0.33	Biweekly	79	61.2
Toxaphene	2.00	Weekly	71	64.2
DDT	1.00	Biweekly	68	68.0
Perthane	1.00	Biweekly	67	69.3
Untreated	—	—	61	70.5
DDT	1.00	Weekly	61	73.5

[a] Range: 20 least damage; 100, greatest damage. Any two means not paralleled by the same line differ significantly at .05 probability by Duncan's multiple range test.

in alfalfa and winter wheat during May and early June (fig. 1). These findings paralleled those of Shirck (1951) and Boyce & Miller (1953) who found that large numbers of onion thrips overwintered in alfalfa and red clover. The fact that the numbers of adult thrips sharply declined in samples of alfalfa and wheat upon the cutting of hay in mid-June and the maturing of wheat in early July (fig. 1.) strongly suggested the time that the adult thrips moved out of these fields.

Peak flights of thrips were evident in the records of thrips observed on tanglefoot traps between June 28 and July 5. These observations and the first appearance of thrips in large numbers the third week of June in the cabbage fields were further circumstantial evidence that alfalfa and winter wheat were important sources of the initial infestation in cabbage. The thrips did not feed extensively nor oviposit on cabbage plants until the leaves beginning to form heads were closely appressed. The time when this occurred varied among consecutive plants in the row. But, if the head was formed and thrips were successfully established within, further insecticidal applications were ineffectual in control.

The relative rank in effectiveness of compounds tested for the control of thrips on cabbage is shown in tables 1 and 2. The loss of marketable cabbage ranged from 36 to 39% of the crop in the untreated check plots in the 1957 experiments. In the best treatment, heptachlor applied weekly at one-half pound per acre, 93 to 94% of the crop was marketable (tables 2 and 3).

The following implications are suggested by the results of this study. (1) Onion thrips infestation of cabbage is a potentially serious threat to cabbage production in view of (a) the wide distribution of *T. tabaci* and of host plants acceptable to this species, and (b) the common use of alfalfa and winter wheat in crop rotations in areas where cabbage is grown. (2) The method of visual rating of relative thrips damage to individual cabbage heads at harvest time provided satisfactory data for evaluating the effectiveness of chemical sprays for the control of thrips infesting cabbage. (3) Heptachlor was superior to other compounds tested for the control of thrips and may offer a practical means of control. (4) Any practices that will insure a uniform rate of cabbage development in the field will facilitate planning for adequate chemical control of thrips since spraying to be successful must begin at initiation of heading before the establishment of thrips within the heads.

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BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

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SCIENTIFIC NOTES

A Method for Studying the Microenvironment of the Spotted Alfalfa Aphid[1]

S. R. Race and R. C. Dobson[2]

During a study of the biology and control of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), in New Mexico, macroclimatic records and observations failed to explain the rapid appearance and disappearance of this insect. Data obtained from this study suggested the need for a thorough understanding of microenvironmental influences on aphid activity and development. Measurements and observations taken from the microenvironment might help explain and predict aphid outbreaks and also the timing of control measures.

Methods.—An electrically powered "Multi-Record Dynalog Recorder"[3] was obtained for this purpose. It was set up and calibrated in the New Mexico A. & M. College entomology greenhouse during January and February, 1957. In March this recorder was fitted into a standard weather kiosk (fig. 1) and placed in a small alfalfa field on the Agronomy Farm of the New Mexico A. & M. Agricultural Experiment Station.

This instrument was designed to take 12 continuous and simultaneous readings adaptable to either temperature or dewpoint. Temperature records were obtained by placing nine "Dynatherm"[3] resistance bulbs at various heights scattered throughout the field (fig. 2). The resistance bulbs were fitted to wooden supports which may be placed at any desired height in the field. Each bulb is about 6 inches long and three-eighths of an inch in diameter. The sensitive element is located in the tip (fig. 3). A galvanized iron shield protects the bulb from the weather. For this investigation, macroenvironmental data were taken at 5 feet above the ground, while the microenvironmental data were taken at several points within the zone of plant growth. In the initial field study, two units were placed at 2, 4, 7, and 11 inches above the ground and one unit at 5 feet above the ground.

The "Dewcel"[3] sensitive element (fig. 3) is about 4 inches long and five-sixteenths of an inch in diameter. A stainless steel sleeve, open at the distal end, protects the sensitive element. Three "Dewcels," fitted to wooden supports adaptable to any

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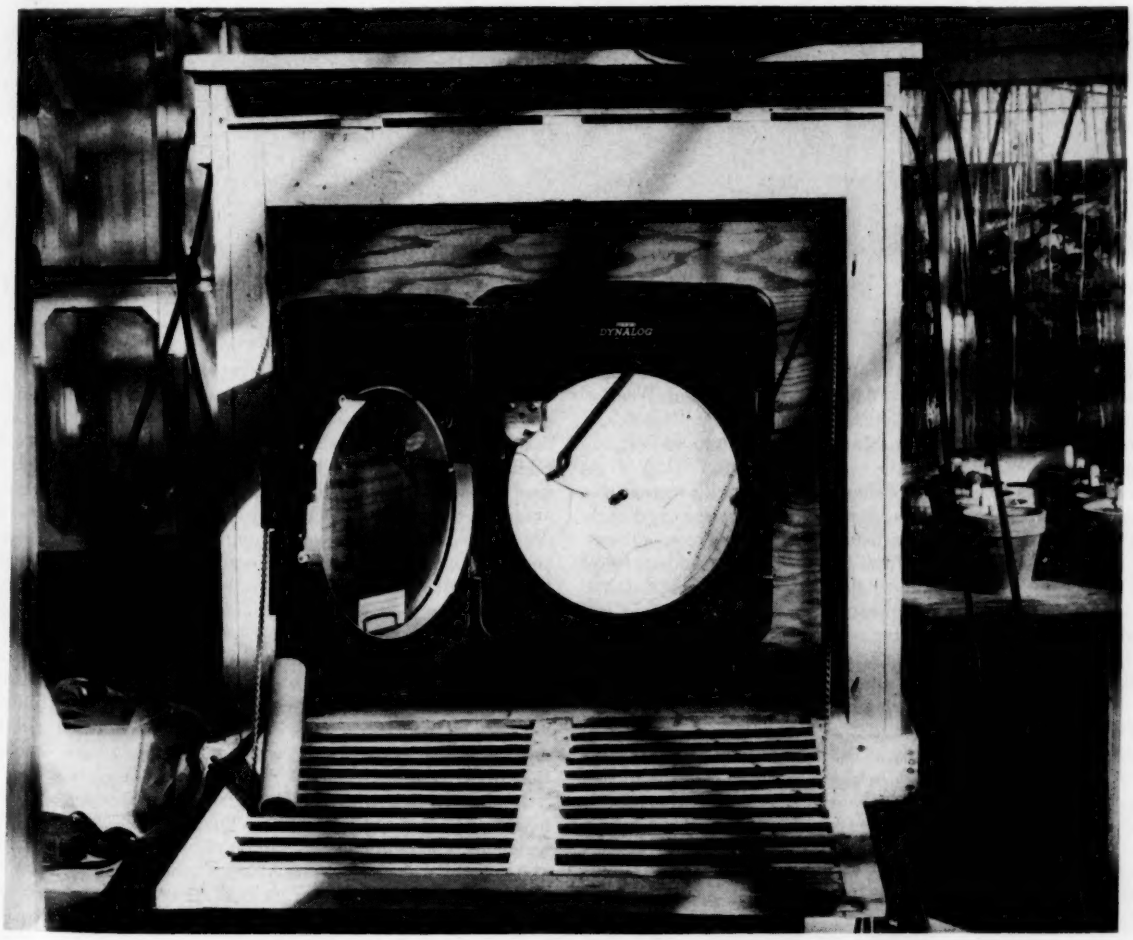

Fig. 1.—A "Dynalog 'Multi-Record' " recorder adapted to fit inside a standard weather kiosk.

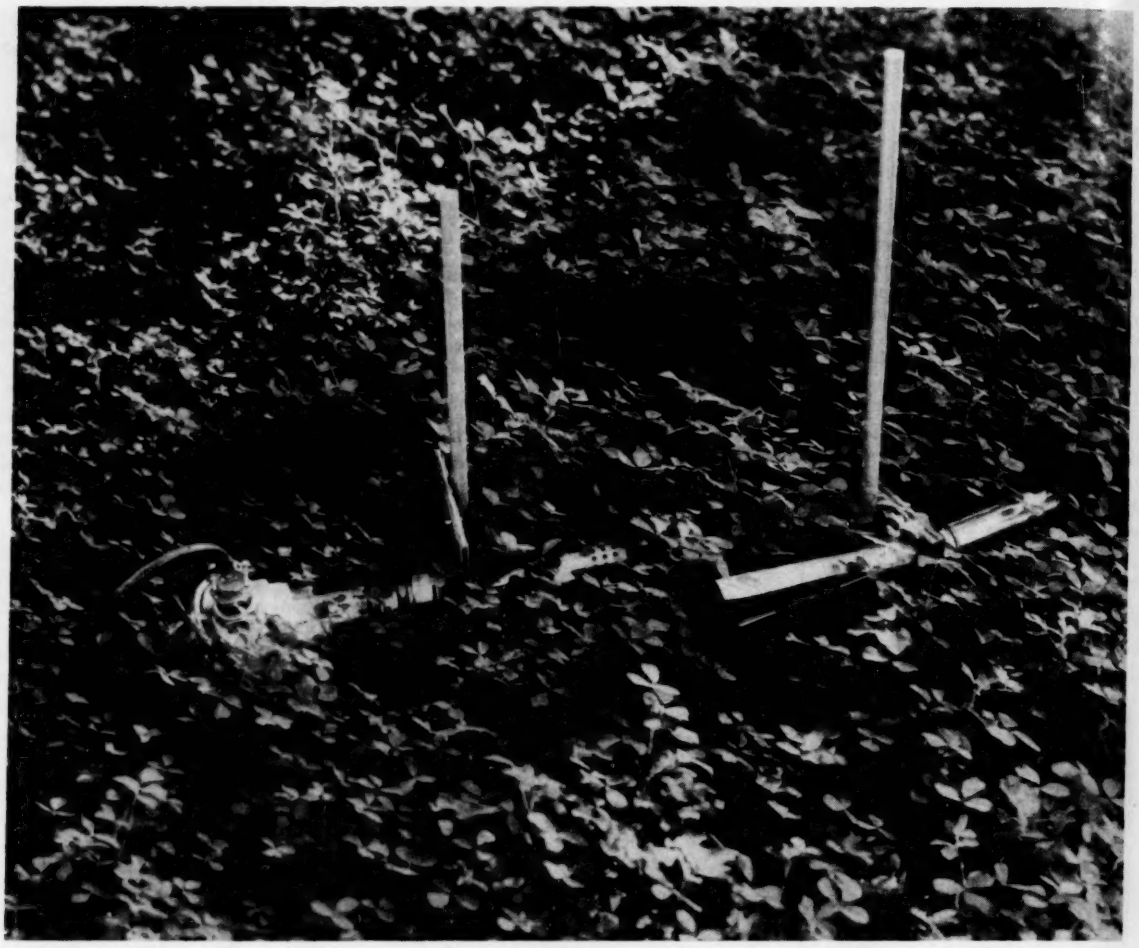

FIG. 2.—A "Dewcel" sensitive element (left) and a "Dynatherm" resistance bulb (right) in an alfalfa field. Both units are 4 inches above the ground level.

desired height, were placed in the alfalfa field (fig. 2). The heights chosen were: 2 and 4 inches, and 5 feet above the ground. Weekly checks on the accuracy of all units, particularly the "Dewcels," were necessary because of splashing mud and rain, and because insects became encrusted on the sensitive portion.

A reading was recorded on a chart every 6 seconds so that the temperature or dewpoint from each location was recorded once every 72 seconds. A continuous field record of these temperatures and dewpoints was obtained from March until November, 1957.

RESULTS.—The data obtained during this study indicated that consistent differences in temperature and relative humidy existed at the various levels studied in the experimental alfalfa field. Figure 4 illustrates the average daily temperatures recorded at each height in the alfalfa. These averages were compiled from a typical week during the growing season.

The temperatures recorded at the 7- and 11-inch heights increased and decreased sharply as the sun rose and set. Temperatures recorded at the 11-inch height were more extreme in this respect than those at the 7-inch height. Figure 4 also reveals that the temperatures recorded from each height in the microenvironment were at their maximum when the sun reached its zenith. During the night, the warmest temperatures in the microenvironment were recorded at the 2-inch height and the coolest temperatures at the 4-inch height. These differences amounted to an average of three degrees. Temperatures recorded at the 5-foot height were consistently higher than those recorded from the microenvironment during the night-time.

Figure 5 illustrates the average daily relative humidities recorded for the same period as shown in figure 4. This chart shows that the average daily relative humidity recorded at the 4-inch height was about 6% higher than that at the 2-inch height. It may be seen, however, that from 9 A.M. to 3 P.M., the relative humidity at the 2-inch height was slightly higher than that at the 4-inch height. The relative humidity recorded at the 5-foot height averaged 36% lower than the average recorded at the 4-inch height.

DISCUSSION.—Although no conclusions were drawn from these preliminary experiments, the data accumulated during the season revealed certain trends with respect to temperature and humidity which could have an important bearing upon the biology and activity of the aphids. The fact that the temperatures recorded at the 7-inch and 11-inch heights increased and decreased rapidly with the rising and setting of the sun indicated that these points were warmed principally by insolation.

It appears that with the exception of the 2-inch and 4-inch heights, the higher the resistance bulbs are placed in the field, the higher the temperatures will be. Temperatures at the 2-inch and the 4-inch heights are reversed in this respect probably because of the increased soil radiation effect at the 2-inch height and a minimum effect of insolation at both heights.

The average daily relative humidity at the 4-inch height was higher than at the 2-inch height primarily because of increased

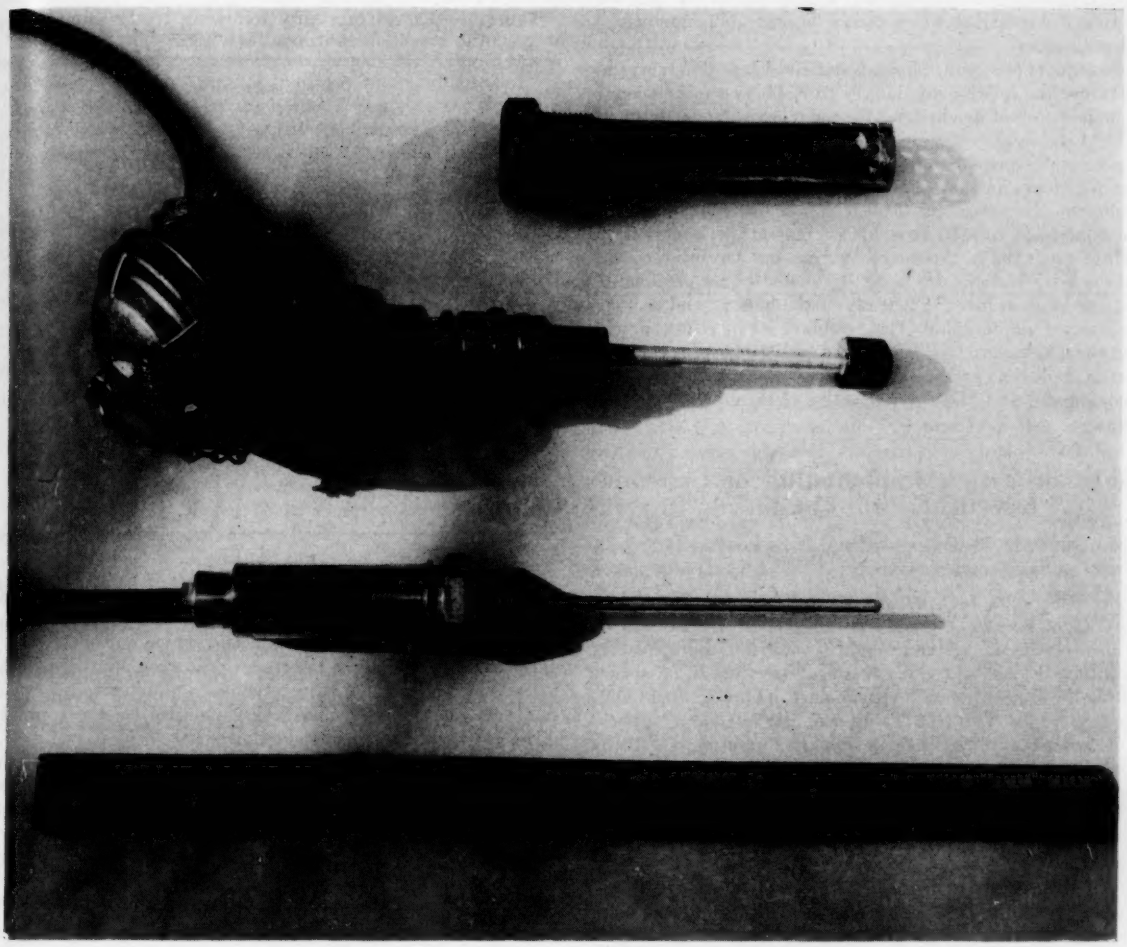

FIG. 3.—A "Dewcel" sensitive element (top) showing the protective sleeve removed. A "Dynatherm" resistance bulb (bottom)

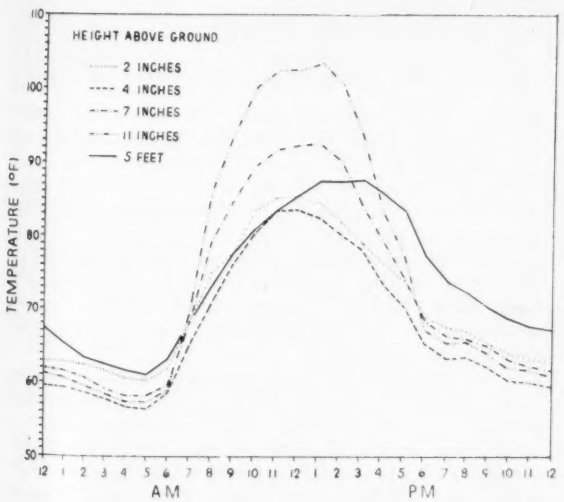

FIG. 4.—The average daily temperatures recorded from the New Mexico A. & M. experimental alfalfa field. These averages were compiled from a typical week during the latter part of the growing season.

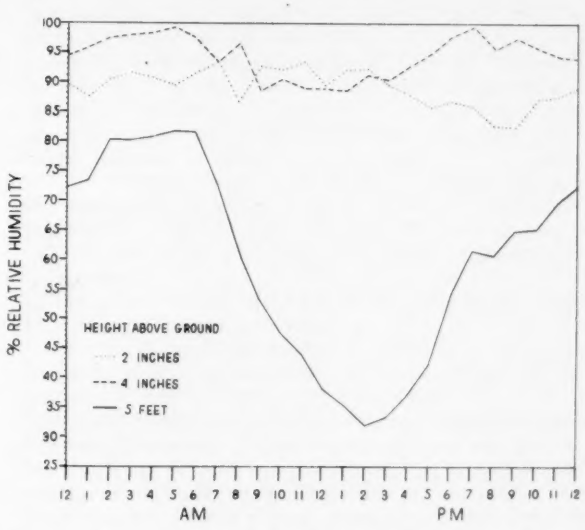

FIG. 5.—The average daily relative humidities recorded from the New Mexico A. & M. experimental alfalfa field. These averages were compiled from the same period as shown in figure 4.

night time soil radiation which causes warmer air temperatures at the soil surface.

The results of this study showed that consistent differences in temperature and relative humidity occurred between the macroenvironment (5-foot height) and the microenvironment (ground level to 11 inches above ground) in the alfalfa field under study.

In general, temperatures, but not relative humidities, recorded in the macroenvironment parallel those recorded in the microenvironment. However, readings from the macroenvironment *per se* should not be used to predict temperatures and relative humidites occurring in the microenvironment. During the winter, these investigations are being conducted under greenhouse conditions as an adjunct to a study of the biology and ecology of the spotted alfalfa aphid. They will be continued in the field during the coming growing seasons. In addition, various shields are being studied as to their heating and cooling effects on the resistance bulbs and "Dewcel" sensitive elements.

Results of Trunk Implantation of Systemic Insecticides in Conifers[1]

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Some success has been achieved using systemic insecticides for control of insects on coniferous trees. Wachtendorf (1954) was unsuccessful in systemic control of *Ips typographus* L., *Ips amitinus* Eichh. and *Pityogenes chalcographus* L. on spruce following the application of methyl demeton impregnated bands to the tree trunks. Vité (1957) with a similar method of application of demeton could neither prevent the formation of galls by *Chermesiden* (*Cnaphalodes*) *strobilobius* Kltb. and *Chermes* (*Adelges*) *abietes* L. nor kill the larvae within the galls. Kinghorn (1955), also using impregnated bands with demeton and schradan, demonstrated systemic control of *Dendroctonus monticolae* Hopk. in lodgepole pine but no control of *D. pseudotsugae* Hopk. in Douglas fir. Methyl demeton impregnated bands were successful for *Dreyfusia nüsslini* C. B. on pine (Vité 1956). On larch both demeton and methyl demeton impregnated bands gave good systemic control of *Taeniothrips laricivorus* Krat., and young larvae of *Coleophora laricella* Hbn., and it appeared as if the methyl demeton was more rapidly translocated than demeton (Vité 1955). Martignoni & Zemp (1956) confirmed the efficiency of this use of methyl demeton for the thrips. Vité (1957) has recently reported that trunk implantation of methyl demeton is efficient in control of *T. laricivorus* and *C. laricella* on larch and that similar use of demeton results in some phytotoxicity. Soil application, insecticide impregnated bands and trunk implantation have been shown effective by systemic action against many pests of deciduous trees (Ripper 1957).

Preliminary studies on trunk implantation of systemic insecticides in conifers were made with larvae of the balsam gall midge, *Itonida balsamicola* (Lint.), and the red-headed pine sawfly, *Neodiprion lecontei* (Fitch) and the results are reported here. Both the midge larvae within their galls and the surface feeding sawfly larvae proved to be convenient bioassay organisms for determining the efficiency of systemic translocation.

MATERIALS AND METHODS.—Five insecticides were implanted into balsam fir, *Abies balsamea* (L.) Mill. The trees were about 4 inches in diameter and 17 feet high. Technical grade insecticides were used with a stated purity of 90% for demeton, Thimet and Chipman R-6199, 50% for dimefox, and 95% for Am. Cyanamid 12880 (*O,O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate). Technical demeton, dimefox and Thimet were introduced directly into the trees, while R-6199 and the 12880 were introduced as 50% and 10% aqueous solutions respectively. The chemicals were implanted in three trees at 4 gm. of the active ingredient per tree and in two additional trees at 1 gm. per tree. Implantation was accomplished by pipetting one-fourth of the dose into each of four holes (½" diameter) located equidistant around the tree trunk and 6 to 12 inches above soil level. The holes were drilled 2 inches deep with a brace and bit at a 45°-angle to the main axis of the tree. Corks were inserted into the holes after introducing the insecticides to minimize pitch exudation and loss of the chemicals. Five control trees were drilled and corked without the introduction of insecticide.

Table 1.—Larval mortality following trunk implantation of systemic insecticides in conifers. 1957.

Insecticide	Date of Treatment	Grams of Active Ingredient per Tree	Days to Per Cent Mortality Indicated		
			50	90	100
		Itonida balsamicola			
Demeton	July 2	4	1.5	3	5
		1	2.5	3	3
Thimet	July 2	4	3	10	14
		1	9	14	—
Dimefox	July 2	4	1	2	3
		1	7	—	—
R-6199	July 2	4	3	71	—
		1	4	—	—
Am. Cyanamid 12880	Sept. 3	4	10	18	26
		1	11	21	26
		Neodiprion lecontei			
Demeton	Aug. 15	4	0.7	1	2
		2	1	2	3
		0.5	4	4.7	5

The tree crowns were divided into the lower third, the middle third and the upper third. Ten galls were removed from each third of the tree and dissected and the condition of the single larva in each gall noted. Larvae failing to move when probed were considered dead. The frequency of sampling infested twigs varied for each chemical and was determined by the rate of larval mortality. The various treatments were sampled up to 16 times at intervals up to 71 days after treatment and the larval mortality in the control trees never exceeded 15%. The kill due to systemic action was calculated as follows:

$$\% \text{ kill} = 100 - 100 \times \left(\frac{\text{No. of living larvae in 30 galls from treated trees}}{\text{No. of living larvae in 30 galls from control trees}} \right).$$

The most efficient systemic against the balsam gall midge larvae was tested against the red-headed pine sawfly larvae. Five colonies each of 10 larvae in the fourth and fifth instar were placed on several 10-year-old red pines, *Pinus resinosa* Ait. Two colonies were placed on an eight-year-old whorl, two on a four-year-old whorl and one on the current year's whorl. Three concentrations of demeton were implanted as previously described. One tree was implanted with 4 gm. of the active ingredient, three with 2 gm. each and one with 0.5 gm. Percentage larval mortality was determined daily. No larval mortality occurred in those trees used as controls.

RESULTS AND DISCUSSION.—Demeton appeared to be the most promising systemic for balsam gall midge larvae as it effected the most rapid and complete kill at the lowest dose (table 1). Dimefox at the 4-gm. dosage per tree gave rapid larval kill, but phytotoxicity was evident after 2 days, and by 10 days, all the needles were yellowed and dropping. Though trees treated with 1 gm. dimefox survived, phytotoxicity was serious enough

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to preclude the use of even this dosage on balsam fir.

Results with the 12880 are not comparable with the other chemicals since it was implanted near the end of the growing season when a reduced translocation rate would be expected.

The Eulophid parasites of the balsam gall midge, the larvae of *Tetrastichus whitmani* (Grlt.) and *T. marcovitchi* (Cwfd.) continued their development within the gall on midge larvae killed by the 12880.

For any given concentration of systemic it was found that the diameter of the tree was directly correlated with the time required for complete mortality of the gall midge larvae. No significant difference in larval mortality was observed at the three levels of the crown.

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Notes on the Biology of *Bruchus brachialis* Fahr.[1]

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The vetch bruchid, *Bruchus brachialis* Fahr., became established in the Middle Atlantic States in 1931 (Bottimer 1931). In Texas, this insect was first observed by C. F. Garner (personal communication, 1953) in Delta County in 1953, and has since spread to most of the vetch-growing areas of northeastern and eastern Texas. This bruchid is currently considered one of the most important economic pests of the crop. Some preliminary observations of the habits of *B. brachialis* under conditions prevailing in New Jersey were reported by Bridwell & Bottimer (1933). Information obtained in a study of the insect in northeast Texas is given in this paper.

The first beetles were collected in sweepings in vetch fields on March 13, which was approximately 5 weeks before the formation of seed pods. The overwintering beetles began oviposition on developing vetch pods during the first week of May, and freshly laid eggs were observed as late as June 20. The number of eggs laid by females isolated on pods of field-growing vetch, ranged from 0 to 19 and averaged 8.5 per day. Under laboratory conditions, 80 to 82° F. and 60 to 70% relative humidity, the average number of eggs laid daily was 5. Eggs were deposited singly on all areas of the pods, and in many instances were found on the inside walls of the cages and vials used for isolating the bruchid. The entire under surface of the egg was securely fastened to the vetch pod with a sticky secretion present on the egg when oviposited. The egg was elongated, flattened and varied in size but was usually between 0.7 and 0.8 mm. in length and the width was approximately one-third its length. The freshly laid egg was pale yellowish green, but soon turned yellowish brown, and was more or less translucent. Each end of the egg was rounded with the anterior extremity slightly larger. The chorion was finely wrinkled and semitransparent. Twenty-four hours after oviposition the interior of the egg formed a fine network of medium brown coagulated material, and on the third day two dark brown spots were noticeable near each end. Although some eggs hatched in 4 days, the majority hatched during the fifth day following oviposition. Upon hatching, the larva ate its way through the ventral surface of the egg shell and then into the green hull of the pod. The larva spent most, and in some instances all, of the first instar making tiny mines in the covering of the immature pod. As the newly hatched larva fed it pushed the frass into the egg shell, resulting in the formation of mottled straw-colored opaque spots. The exit hole in the egg shell was always at the broad end of the egg, and the entrance hole in the hull of the pod was directly beneath it. Of 297 larvae removed from seeds, in only a few instances were there more than one individual found per seed; and these larvae had not developed beyond the second instar. Frequently, more than one larva was found in the feeding mines in close proximity of a developing seed

Table 1.—Body and head measurements (in mm.) of *Bruchus brachialis* Fahr.

Stages	Body Measurements: Number Measured	Body Measurements: Mean Width	Body Measurements: Mean Length	Width of Head Capsule: Min.	Width of Head Capsule: Max.	Width of Head Capsule: Mean	Width of Head Capsule: Number Measured
First instar	14	0.29	0.67	0.9	0.16	0.13	45
Second instar	20	0.53	1.23	.23	.32	.27	59
Third instar	53	0.86	1.68	.34	.48	.41	89
Fourth instar	62	1.40	2.57	.50	.68	.55	104
Pupa	43	1.56	3.24				
Adult male	50	1.84	2.92				
Adult female	50	1.90	3.01				

Based upon the measurements of the head capsule, the vetch bruchid has four larval instars (fig. 1 and table 1). The measurements were taken across the widest section of the head capsule using an eyepiece micrometer adapted to a stereoscopic microscope. The larvae, pupae and adults were taken from samples of vetch pods, which were collected on several dates, from the time the pods began to form until the vetch was harvested. Body measurements of the larvae, pupae and adults are given in table 1. Immature stages of the bruchid were kept in a fixing solution for 12 to 24 hours before preserving in 70% alcohol. No attempt was made to straighten the curved body of the larva, consequently, the mean length recorded was comparatively less than those for the pupa which was less-curved in normal form (table 1). All measurements of immature stages were made from a dorsal view. The wing pads of the pupa were not included in width measurements. In case of the adult, the lateral aspect was used for length, the ventral view for width measurements, and the greatest extremities were measured in each case.

The larvae varied considerably in size, particularly in the third and fourth instars (1.1 to 3.5 mm. and 1.4 to 3.7 mm., respectively), and a similar variation was found among specimens of both the pupal and adult stages. Also, a great variation occurred in the width of the head capsule of the fourth-instar larvae (fig. 1). Since one vetch seed accommodates the complete development of only one individual, it is apparent that the amount of food supply in small and large seeds would determine, to some extent, the size of the insect.

The time required for the development of the bruchid from eggs laid on June 10, to the emergence of adults ranged from 22

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[2] Associate Professor and Technical Assistant, respectively.

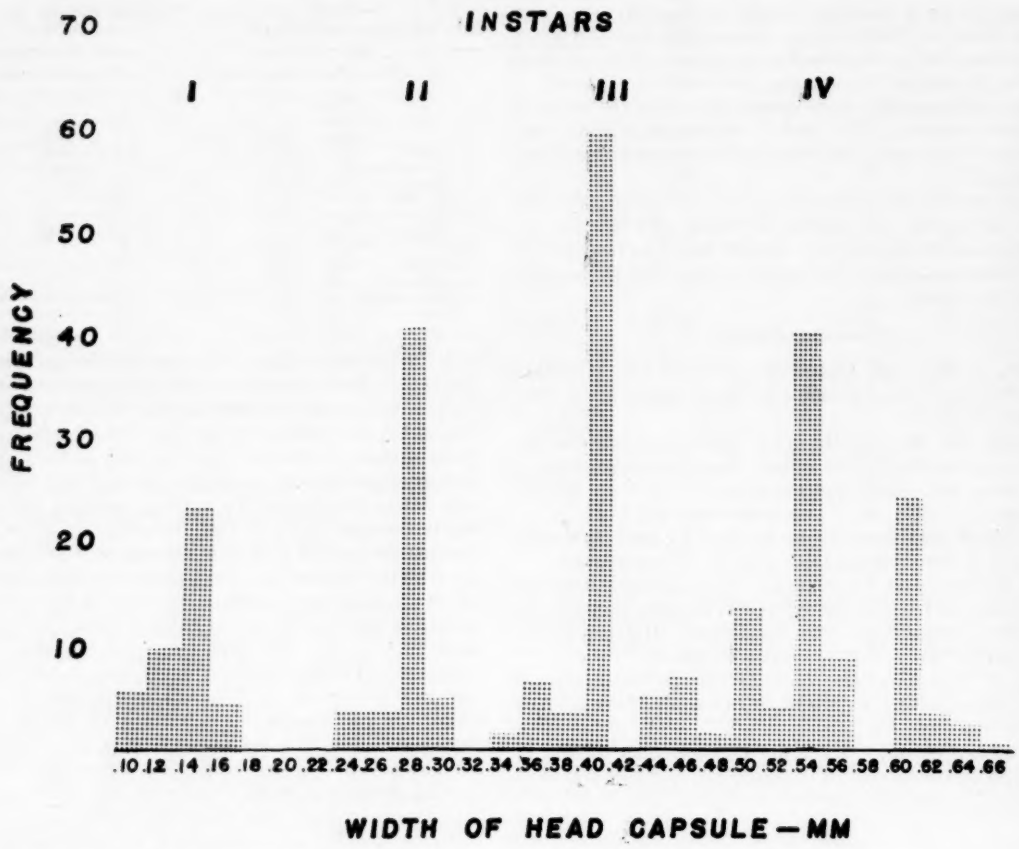

FIG. 1.—Width of head capsules of the four larval instars of *Bruchus brachialis* Fahr.

to 43 days. with an average of 30 days. Only one generation occurred during the year.

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Crambus calliginosellus Clem. Destructive to Carrots in Western New York[1]

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Since its description in 1860 (Clemens 1860) the corn root webworm, *Crambus calliginosellus* Clem. has repeatedly been cited as a pest of economic importance. In this connection it has been associated with injury to corn (Beckwith 1891) or tobacco (Johnson 1899) and is known in local areas by a variety of names reflecting the host involved and the type of injury incurred (Runner 1914). Although Felt (1894) reported this insect common near Ithaca and Sanderson (1902) described its injury to tobacco and corn, the species has not been recognized as an important economic pest in New York State.

During 1955 a late July inspection of highland carrots in western New York revealed a considerable amount of injury on the edible root by the feeding of a lepidopterous larva. The larvae involved were tentatively identified as *Crambus sp.* and later, upon examination of adults reared from these larvae, a *Crambus calliginosellus* Clem.

Injured roots are shown in figure 1. The amount of injury varied from minor feeding marks to excavation of an entire side of a root. Normally feeding was initiated about one-half inch below the soil surface and during this time the larva remained encased in a tubular, web-lined gallery fastened to the surface of the carrot. Seldom did feeding penetrate to the central core of the root. Instead the larva would feed in the tissues immediately below the epidermis and there excavate a cavity of variable size, leaving the epidermis intact except for the entrance area. Usually the entrance to the feeding cavity was covered with webbing and a considerable amount of webbing and frass surrounded the larva in the root. Soil particles enmeshed in the webbing and fungus growth often made infested roots quite repulsive. In some cases the gallery of a single larva was fastened to several carrots along a row and each root had been fed upon to a greater or lesser extent. The amount of injury increased as the season advanced and in one field approximately 20% of the roots had been damaged prior to harvest (fig. 2). Inspection of other fields indicated 3 to 5% injury in highland carrots in western New York while only a trace was found in carrots grown in muck areas.

During 1956 and 1957 routine inspections for this pest have been made in western New York. In neither year has injury in

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[2] The writers wish to thank Mr. H. W. Capps, United States Dept. of Agriculture, Insect Identification and Parasite Introduction Laboratories, U. S. Department of Agriculture, for identification of the insect species involved.

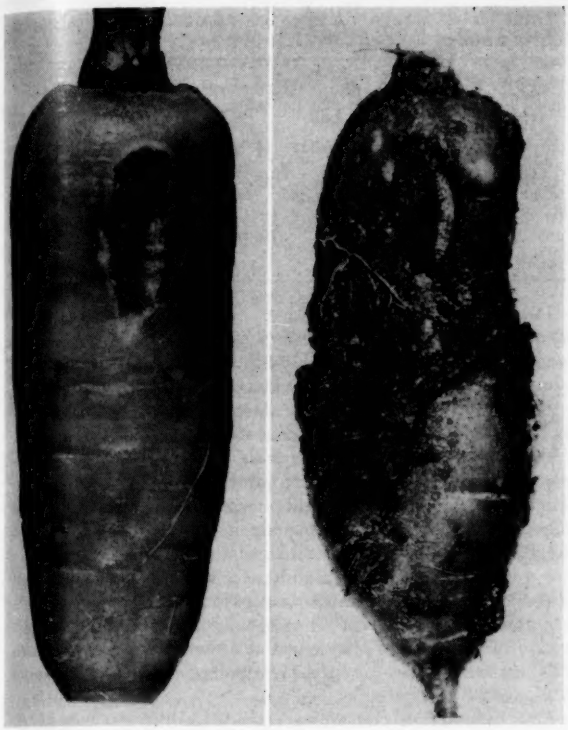

FIG. 1.—Moderate (left) and severe (right) injury to carrots by the corn root webworm, *Crambus calliginosellus* Clem.

excess of 1% been found in carrots. In most fields examined injury caused by this webworm has not been found. The status of this insect as a pest of carrots is, therefore, uncertain; however, its ability to cause appreciable injury to this crop must be recognized.

In an extensive study of *C. calliginosellus* Clem., Runner (1914) reported buckhorn plantain and oxeye daisy as preferred hosts of the insect in Virginia and that several other weeds, including wild carrot, *Daucus carota*, were also acceptable food plants for the developing larvae. In view of this it is not surprising to find these larvae feeding on the cultivated carrot: however, the author is unaware of any previous report in this connection.

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FIG. 2.—Injury to carrots by the corn root webworm: 100 carrots selected at random from a badly infested field, divided into injured (left) and uninjured (right).

Rufous Scale on Robusta Coffee in Ecuador[1]

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Robusta coffee is heavily infested with rufous scale, *Selenaspidus articulatus* (Morg.), in most localities where it is grown in any quantity. It attacks the upper surface of the leaf almost exclusively as stated by Yust & Cevallos (1954–55A). The scale also attacks Arabica coffee but the population density seems to be less on Arabica than on Robusta where the two varieties are adjacent. Some large acreages of Robusta coffee are being planted now in the coastal area. Since the rufous scale turns the leaves yellow, defoliates the coffee bushes and greatly reduces production it may become very important in the production of this type of coffee in Ecuador.

POPULATION CHARACTERISTICS.—At the Tropical Experiment Station, Pichilingue, there are a number of adjacent plots of Robusta and Arabica varieties of coffee planted at the same time from seed and it appears that both are equally exposed to opportunities for infestation by rufous scale. To learn whether there was a real difference in susceptibility to infestation, population counts were made on leaves of the two varieties in five adjacent plots. The average population per leaf on Robusta was 47 scales and on Arabica 2.2 scales. The Robusta leaves are larger than the Arabica leaves so the counts were transposed to a basis of 100 square centimeters. In this case Robusta had an average of 35.2 scales per 100 square centimeters of leaf surface against 5.3 scales per unit area on the Arabica coffee showing definitely that Robusta is more heavily infested with scale than Arabica varieties. A survey of 80 Robusta bushes was made at the Tropical Experiment Station to determine population distribution in the field. Counts were made of the number of scales per leaf and were grouped in two ways, namely, (1) three adjacent plants and (2) each 10th plant in groups of three plants. With the first method, the coefficient of variation averaged 61% and with the second it averaged 36%, suggesting that the infestation tends to be concentrated on adjacent groups of bushes and is not uniformly distributed as might be expected if the infestation was distributed on a chance basis, as by wind. This suggests that ants may be involved as they are ubiquitous in a coffee planting.

CONTROL.—In order to test several treatments for control of rufous scale a planting of over 600 young Robusta coffee bushes was divided into eight blocks. Two blocks were treated with each of three treatments at random on February 4, 1955, as follows:

Oil 2%
15% parathion wettable powder, 3 lb. per 100 gal.
1.5% oil plus 15% parathion wettable powder 1.5 lb. per 100 gal.

Blood albumen was used with the parathion alone at the rate of ¼ pound per 100 gallons of water. Approximately 0.4 of a gallon of spray mixture was applied per bush from two sides with a pressure of 350 p.s.i. which resulted in good coverage. The above treatment was repeated on February 24, 1956. An inspection of the trees was made on March 8, 1955, during which time both mortality and population counts were made. To make the former, heavily infested leaves were selected from each of 10 trees from each block and 20 mature female scales on each leaf were carefully inspected to determine whether they were dead or alive. Hence, each mortality record was based on a count of 400 insects. The percentage of kill was calculated and corrected for natural mortality. Population counts were made at irregular intervals. Those made in 1955 consisted of 20 units of three leaves each, one each per bush, or one leaf each from 60 bushes on which all adult female scales, dead and alive, were counted. To select the leaf for counting, the bush was circled and a representative heavy leaf was chosen. The bushes from which the leaves were selected were taken at random. The system was changed in 1956 so that the unit was based on 12 leaves per three bushes and there were 10 units per block. Four old leaves were selected from each bush, one from each of the four aspects of the tree, about shoulder high but the leaves were selected at random as far as scale population was concerned. Again the count for each treatment was based on leaves from 60 bushes. The corrected percentage of mortality for the three treatments was as follows: oil, 57.1; parathion, 74.4; oil plus parathion, 97.0. The combination oil plus parathion gave the highest kill. A difference of 18.8% was significant at the 1% level so the difference between the combination treatment and the other two was highly significant. A difference of 14.0% was significant at the 5% level so that the mortalities for each of the treatments was significantly different. It is not surprising that the combination treatment was very effective because it gave very high kills of purple scales on citrus as reported by Yust & Cevallos (1954–1955B).

Table 1.—Average number of adult female scales on Robusta coffee leaves.

TREATMENT	AVERAGE NUMBER OF SCALES PER UNIT			
	1955			July 3, 1956
	March 8	July 23	Oct. 23	
Oil	13.9	12.6	51.8	9.9
Parathion	10.2	14.0	47.4	9.2
Oil plus parathion	11.9	14.2	62.4	9.6

Four population counts were made in the various blocks throughout the study with results as shown in table 1. The scale population did not differ significantly between treatments at any time, indicating that all three treatments controlled the rufous scale. According to the October count the population increased significantly in all three treated areas, which would be expected. These results suggest that oil alone was much more effective than indicated by the mortality count probably because it has a longer residual action and prevented crawlers from becoming established although many adult females were not killed. Parathion alone also gave effective control which may be due to a much higher kill of younger stages. Earlier tests with 1% oil plus 1.5 pounds of 15% parathion w.p. in 100 gallons of water showed that this dosage was not effective. As a result of considerable experience, it now appears that control can be obtained with an average of two treatments in 3 years using 1.5% of oil plus 1.5 pounds of 15% parathion w.p. in 100 gallons of water.

The treatments with oil or with oil plus parathion caused slight damage to immature tender terminal leaves which were less than 3 cm. long but it was not serious.

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A Poison Bait for the Control of *Leptocorixa acuta* (Thunb.)[1]

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Leptocorixa acuta (Thunb.) (Coreidae: Hemiptera) is one of the most important pests of rice in Indonesia; the author estimates that it reduces the national rice yield more than 10%. It is probably the most important pest of rice in upland rice areas and it also causes damage in lowland flooded areas. In many areas 100% loss of yield is noted; large areas record losses of over 25%. *L. acuta* is the most important of eight species of this genus found in Java (Van Der Goot 1949). Until recently there has been no adequate control recommended which was within practical reach of the small farmer. Area treatment by chemical control is too expensive both from the point of view of the insecticide and the equipment.

Former control methods included lighting fires at night to attract the adults; other methods included burning twigs of rubber trees windward of the infested fields. Prevention of economic loss by hand collection was rarely undertaken. A common method was baiting the insect with certain rotting plants or decaying animal matter. Early in the morning the farmer could collect thousands of adults from a pile of this rotting bait.

The inadequate bait method formerly used was modernized, and adequate control can now be obtained at very low cost. A crustacea (*Potamon* sp.) which makes holes in the walls enclosing the flooded field and also attacks the rice plants was found to be an ideal bait. These crustacea are collected, tied with a string, dipped in 0.2% dieldrin suspension, and each is tied near the head of a flowering rice plant. Two hundred or more bait stations (a maximum of 7 meters apart) per hectare are recommended.

Baits become attractive from a few hours to 2 days after placement in the field. This bait will continue to be attractive for a period of 10 days to 3 weeks; attractiveness will continue for the longer period if rains or heavy dew occur. The adults attracted to this bait are mostly males at a proportion of 5 to 1. However, use of more than 200 baits per hectare with an adequate contact insecticide can eliminate the entire population from a field in a single night.

Other baits that can be used are rats, pieces of fish, other species of crustacea, and African snail, *Achatina fulica* Fer. Crushing of the shell of the snail is necessary for putrefaction to begin rapidly; this is not necessary with the crustacea. The size of the bait is not important; a large number of smaller baits is better than a few, widely scattered large baits. Some plants, such as *Hydrilla verticillata* Presl., can be put in a small burlap bag which has been dipped in dieldrin, or the plants themselves can be dipped in the insecticide solution and wound around the stalk near the flowering head without the use of a bag.

A small sack made from a 6- to 8-inch square of any material such as used burlap can be dipped in dieldrin 0.2%, filled with animal bait, and tied near the rice head. This method keeps the bait damp a little longer and provides a bigger surface area; sacks can be collected, refilled, and moved to new fields. The use of sacks required more insecticide and, therefore, usually a higher cost for materials.

This method of using a large number of baits is very laborious, but it fits into the Indonesian need of *Leptocorixa* control, avoidance of cash expenditures by the farmers, and limitation of the use of foreign purchased insecticides.

For a 1-hectare area, control based on the use of crustacea requires about 20 grams of 50% dieldrin wettable powder and 20 grams of tapioca flour used as a sticker; these are mixed with 4 liters of water. These chemicals cost the farmer the equivalent of 1 kilogram of rice.

The labor required to collect, tie and dip 200 to 500 baits such as the crustacea was much less than the time required to dust or spray the entire field with hand-operated equipment. The maximum amount of time needed in Indonesia for this baiting work was 2 man-days, most of it used for collecting the crustacea. The cost of one day's farm labor on Java is between 2 and 3 kilograms of rice, or about the same as 60 grams 50% dieldrin wettable powder purchased by the farmer. Experiments are now being carried on with a fish meal-talc dilution including a low concentration of contact insecticide. Wet fish meal is also attractive to the *Leptocorixa* adults. To date this test was only for experimental data because the cost of a kilogram of fish meal is far beyond the cost of 200 fresh baits.

The distance of attraction of these rotting baits seems to be less than 2 yards; yet the frequent movement of the adults insures their arrival at a point within that range when the baits are regularly spaced within the field.

The adult usually attempts feeding when alighting on the bait. In various countries affected by *Leptocorixa*, poison baits consisting of rotting animal material and calcium arsenate have been used in the past. This method of baiting has been unsuccessful and is not now generally used in any of these countries, probably because not enough individual bait stations were used and because the range of attraction is only a few meters.

Leptocorixa feeding on the milk stage of the rice seed results in an empty seed pod. Each adult can be estimated to damage more than one pod in a day. The period of attack on a single plant is less than 5 days; for an average field in which initiation of flowering is spread, the main period of attack will be about 10 days. On this basis even one adult per square meter will result in a loss of 10,000 grains of rice per day; 30,000 grains weigh 1 kilogram. Normally farmers do not consider attack to be serious until there are populations of over 20 per square meter.

The low cost of this method of control makes it worth while for a farmer to use it if he finds a population of only one *Leptocorixa* per square meter. Since most farmers work an area of only about one-half hectare and this is often planted over a period of 2 to 3 weeks, the work requirement for any plot coming into flower will be less than one day's work for the farmer.

This method has only recently been introduced to the farmers of Indonesia. The fields where it has been tried have indicated its efficacy. However, there has been so far a limited application of the method because of the difficulties in informing the farmers and because insecticide is inadequate.

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Notes on the Biology and Control of *Fiorinia externa* Ferris[1]

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According to Ferris (1942) this armored scale was first reported by Sasscer from Queens, Long Island, New York under the name *Fiorinia japonica* Kuwana. It was later described by Ferris (1942) as a new species from material collected in Maryland by H. S. McConnell. Subsequent collections by McConnell have shown this diaspid is a local problem in the area of Towson, north of Baltimore. Identifications of this species by Dr. Harold Morrison, Entomology Research Division, U. S. Department of Agriculture, have shown it is also present in Connecticut, Pennsylvania, Ohio, and New Jersey.

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FIG. 1.—Ventral view of hemlock twig showing whitewashed appearance caused by developing scales. Dark spots are adult female coverings, white spots are male coverings.

Fiorinia externa Ferris infests primarily hemlock and secondarily yew and spruce in Maryland. Infestations of this scale are commonly very heavy wherever found. Infested hemlocks take on a mealy, whitewashed appearance (fig. 1), especially in the spring and fall, which decreases their decorative value.

Unlike most female armored scales members of this genus live as adults within the cast skin of the last molt, a condition which Ferris terms "pupillarial." The female contracts upon completion of the last molt, thus occupying only the anterior half of the cast skin. The remaining space is filled with eggs which are laid in two rows with their ends meeting in the median longitudinal axis of the cast skin. The average number of eggs at one time appears to be six. As the crawlers hatch and escape from within the cast skin, more eggs are laid. Chorion counts taken from beneath the coverings of living egg-laying females, which were removed from the previous year's growth, showed that thus far an average of 20.4 eggs had been laid. Longevity has not been determined.

Upon their escape from the female covering, the crawlers are very active. They usually settle within 48 hours on one of the two white stripes which parallel the midrib on the ventral surface of the needle. Once settled the crawler begins to secrete two types of waxy coverings. From the posterior end a rough, frosty-appearing layer is secreted which eventually covers the entire first instar. Simultaneously, two long waxy threads are secreted from a pair of glands located between the antennae on the anterior margin of the head. The latter builds up as a mass of tangled, trailing, white threads and is responsible for the mealy appearance of infested trees.

Monthly field observations have shown that active crawlers and all developmental stages of both males and females are present throughout the entire year. Greatest reproductive activity, however, occurs in the spring and fall in Maryland.

Because it is capable of reproducing throughout the year, and because females continue to produce and protect crawlers over a long period of time, insecticidal control has thus far been unsuccessful. Malathion-DDT mixtures gave excellent crawler kills, but the number of applications needed makes their use impractical. Work with systemics to kill adult females has proved fruitless as of this writing.

Evidence of parasitism was found on all field-collected material. Rearing studies produced chalcid wasps of the genus *Prospaltella*. Examinations of the total female population of a collection have shown that parasitism may be as high as 16.6%.

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A New Technique for Rearing Carpenterworms[1]

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One of the problems in rearing insects under artificial conditions is that of providing a favorable environment in the laboratory. Primary wood boring insects are especially difficult to

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FIG. 1.—Glass cage filled with water-agar-sawdust medium for rearing and studying carpenterworms.

FIG. 2.—Larva in tunnel as viewed through the side of the glass cage.

rear because they bore in living trees. When sections of a tree containing larvae are brought into the laboratory, drying, shrinking, and splitting of the wood may kill the larvae. Furthermore the larvae cannot be observed when they are in the tree or bolt.

A technique using an artificial medium enclosed in a glass cage has been developed in connection with research on the biology of the carpenterworm (*Prionoxystus robiniae* (Peck)). This "sandwich" or "woodburger" technique has promise as a method for rearing wood boring insects for use in research, demonstrations, and teaching.

The cage (fig. 1A) is made of ordinary window glass or transparent sheet plastic glued together with a clear glue. Cages 10 by 6 by 0.7 inches are satisfactory but dimensions may be varied as desired. The top end of the cage is left open until the medium is placed in the cage. A hole 0.7 inch high is left in one edge of the cage about 1.5 inch from the bottom for the entrance of the larvae and the ejection of frass.

The medium consists of 290 grams of sawdust thoroughly mixed in a hot solution of 20 grams of agar in 1 liter of water. Sawdust is taken from the species of tree in which the larvae are found and is kept frozen in a plastic bag until used.

The hot medium is placed in the cage and packed to exclude air pockets. An aluminum foil plug is taped over the entrance hole while the cage is being filled. After the mixture has cooled to room temperature, the aluminum plug is removed and the larva placed in a small 14×18 mesh wire basket fastened over the entrance hole with transparent tape. The top of the cage is then covered with screen wire, transparent tape, and aluminum foil to prevent escape of the larvae and to conserve moisture. A hole dug about 0.5 inch into the medium makes it easier for the larvae to enter the cage.

Glass sides of the cage permit observation of the larva and the upright position allows near normal movement. The larva tunnels through the medium and extrudes frass into the wire basket (fig. 1). It makes clear-cut tunnels (fig. 2) similar to those made in a tree. The medium apparently provides the necessary food and moisture. In some cases, after the larvae have made excessive tunnels through the medium, it may be necessary to add more water. As of January 1958, larvae have lived in these cages for 41 days without apparent reduction in activity. Some of the larvae have sealed the entrance holes with frass. Whether they will pupate and complete their life cycle remains to be seen.

Bayer 21/199 as a Deterrent to Screw-Worm Attack in Sheep[1]

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Incidental to the work done at Kerrville, Texas, with Bayer 21/199 as a systemic insecticide, it was found that the compound was an excellent contact insecticide against screw-worms (*Callitroga hominivorax* (Cqrl.)). Kaplanis and co-workers (unpublished) found that the radioactive compound moved from treated to untreated parts of a calf's body very quickly in spite of efforts to prevent such movement. Young screw-worms in an open wound died a few hours after implantation, and radioassays of the wound and the larvae indicated much higher concentrations of insecticide than was found in the blood of the animal. Larvae developed normally in another wound on the same animal that was protected from external contamination by a gauze bandage.

Small-scale tests with artificially infested sheep and cattle indicated that the compound was highly toxic to all ages of fleeceworm larvae (principally *Phormia regina* (Meig.) and *Callitroga macellaria* (F.)) and screw-worms. All the larvae present at the time the animals were sprayed were killed within a few hours, and it appeared that excellent protection against reinfestation was afforded by several concentrations.

More extensive tests were therefore undertaken to evaluate the compound as a protective agent under field conditions using animals that are subjected to screw-worm attack. In the sheep-raising areas of Texas most of the shearing is done at a time when screw-worm flies are very active. Angora goats are also exposed to screw-worm attack at the time of the second shearing in the late summer or early fall. Ranchers ordinarily treat the severe shear cuts with EQ-335 smear (3% lindane wound dressing) or some proprietary remedy, such as docking fluid, before the animals leave the shearing pen, to protect the cuts from screw-worm invasion until they are healed or at least until they are covered by a dry scab. In addition, the sheep are usually inspected several times in the 2 to 4 weeks following shearing, so that all infestations will be detected and treated before the animal is killed by screw-worms.

Arrangements were made with several Kerr County ranchers to spray their sheep with Bayer 21/199 immediately after shearing. Flocks were divided so that concentrations of 0.25% and 0.5% applied as sprays could be compared with EQ-335 smear. The sheep were sprayed in small pens in groups of 50 or less, with a power sprayer operating at a pressure of 250 to 350 pounds per square inch. About 1 quart of spray was applied to each animal. Recommended practices of the U.S. Department of Agriculture were followed in the treatment with EQ-335. A total of 11,065 sheep were treated to protect shear cuts from infestation, and 408 sheep and goats were treated to protect other predisposing causes, such as injuries associated with lambing, castration, docking, ear tagging, and old screw-worm wounds. These animals were distributed among nine ranches.

Most of the sprays were suspensions that had been prepared

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Table 1.—Protection of sheep and goats from screw-worm attack with Bayer 21/199 sprays. Kerr County, Texas, 1957.

Date	Ranch	0.5% Spray		0.25% Spray		Smear EQ-335	
		Number of		Number of		Number of	
		Animals	Screw-worm Cases	Animals	Screw-worm Cases	Animals	Screw-worm Cases
		Treatment of shear cuts					
May 17	Barker	140 sheep	0	—	—	40 sheep	2
May 9–14	Kott	87	1	—	—	105	3
May 24 to June 11	A. Real	—	—	2231 sheep	21	991	22
May 2	Neunhoffer	157	0	—	—	163	5
May 25 to June 4	F. Real	939	—[a]	312	14[a]	1200	60
June 5	McNutt	423	7	164	0	935	47
June 18–25	Syfan	—	—	889 lambs	11	80	13
June 7	L. Real	598	0	511 sheep	0	1100	80
Subtotals		2344	8	4107	46	4614	232
		Predisposing causes other than shear cuts					
May 15	Kott	3 lambs	1				
		2 kids	0				
April 30	A. Real	—	—	78 lambs	40[b]	100 lambs	6
June 24	YO Ranch	40 sheep	0	—	—	—	—
April 30	A. Real	—	—	100 ewes	0	—	—
		—	—	38 sheep	1	—	—
June 5	Syfan	—	—	12 goats	0	—	—
		—	—	35 sheep	1	—	—
Subtotals		45	1	263	42	100	6
Total		2389	9	4370	88	4714	238

[a] Records for 0.5% and 0.25% sprays were inadvertently pooled.
[b] These lambs were completely protected until they were exposed to a very heavy rain 10 days after treatment.

from a 25% wettable powder. A small portion of the animals was sprayed with emulsions, but no evidence was obtained that the different formulations gave different results.

Most of the records on the incidence of screw-worms in the treated animals were furnished by the owners, as it was not feasible to move the animals into pens for close inspection at frequent intervals. Therefore, the records listed in table 1 are not considered to be exact, but very close approximations.

The tests were conducted during a period of heavy rainfall, and many of the test animals were exposed to heavy rains. Screw-worm flies were abundant and active at all the locations, and all the treatments were exposed to severe reinfestation pressure.

RESULTS.—In general, the sprayed animals were protected from infestation for 2 to 3 weeks, which was adequate time for most of the injuries to heal, either completely or until they were no longer attractive as an oviposition site. Reports from the ranchers indicated that whenever a spray treatment failed it was at least 10 days after treatment. On the other hand, it was not uncommon for the EQ-335 to fail within 2 to 4 days.

Since it was not practical to respray animals that became infested, all animals were considered out of test as soon as they were found to be infested. Records of the number of re-treatments were not obtained, but several ranchers reported that the screw-worm outbreak was so severe that EQ-335 had to be applied every 2 days until the infested wounds healed.

The data are presented in table 1. The incidence of screw-worm infestations in each treatment group can be expressed as number of cases per 1000 animals as follows: 0.5% spray 3.8; 0.25% spray 20.1 and EQ-335 smear 50.4.

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Fumigation Properties of Dow ET-57[1]

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A lindane-impregnated Fiberglas screen placed in the air-conditioning system of aircraft was found to release sufficient vapor to act as an efficient fumigating agent at lindane-in-air concentrations of less than 0.2 p.p.m. (Quarterman & Sullivan 1953). However, even these low concentrations may be considered hazardous. Therefore, a further search (Ihndris & Sullivan, unpublished data) was made to find an insecticide harmless to man and animals which can be used as a fumigant in a similar manner. Dow ET-57 was selected as one of the insecticides worthy of investigation for this purpose. The fact that this compound has a relatively low acute mammalian toxicity and a vapor pressure of 0.0008 mm. Hg at 25° C. (Dow Chemical Co.) indicated that the concentration in air might be so low as not to constitute a safety hazard.

In order to approach actual field conditions in studies concerned with disinsectization of aircraft, the fuselage of a dismantled C-47 airplane was set up at Beltsville, Md., outdoors in an unshaded area. In the spring of 1957 tests were conducted within this fuselage, which has a volume of approximately 1800 cubic feet. Data were obtained on its fumigating effect against several insects and its concentration in air.

A Fiberglas air-conditioning filter, 16×20×1 inches, impregnated with 25 grams of Dow ET-57 was mounted on a 16-inch oscillating fan placed near one end of the fuselage. Normal air exchange was allowed to take place with the doors shut. To attain conditions close to equilibrium, the fan was turned on 2 hours before an experiment was started.

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Table 1.—Effectiveness of Dow ET-57 against various insects when dispersed in a C-47 fuselage by blowing air through a treated filter.

TEMPERATURE (°C.)	PER CENT KILL OF CAGED INSECTS				PER CENT KNOCKDOWN OF FREE-FLYING INSECTS	
	1-Hour Exposure		4-Hour Exposure			
	House Flies	Mosquitoes	Cockroaches	Flour Beetles	House Flies	Mosquitoes
16–18	87	—	—	—	80–90 in 2 hours; all in 3 hours	—
22	100	—	—	—	Started down in 30 minutes	—
25–27	100	100	19	53	All in 1 hour	95 in 3 hours
25–30	100	100	53	95	All in 1 hour	80–90 in 2 hours
24–33	100	100	77	100	All in 1 hour	95 in 3 hours
33–46	100	100	100	100	All in 1 hour	100 in 1 hour

From 500 to 1000 free-flying house flies (*Musca domestica* L.) and a like number of yellow-fever mosquitoes (*Aedes aegypti* (L.)) were exposed to the vapors in each test. Knockdown counts were made in 0.5, 1, 2, and 3 hours.

In addition, house flies, mosquitoes, and cockroaches (*Periplaneta americana* (L.) in 3×8-inch cylindrical screen cages and flour beetles (*Tribolium confusum* Duv.) in petri-dish halves were placed at five stations in the fuselage. The flies and mosquitoes were removed after being exposed to the vapors for 1 hour, the cockroaches and flour beetles after 4 hours. They were placed in a holding room, and survival was determined in 24 hours for the flies and mosquitoes, and in 96 hours for the cockroaches and flour beetles.

Preliminary experiments indicated that a sampling procedure similar to the one developed for the determination of lindane in air (Hornstein & Sullivan 1953) could be used for ET-57. The air sample was aspirated through an absorption column containing 10 grams of alumina, by an air pump in series with a dry gas meter. The usual volumes measured were 10 to 20 cubic feet. Sampling times were 0.5 to 1 hour.

The ET-57 absorbed on the column was eluted with anhydrous methanol, the methanol gently removed on a steam bath, and the residual ET-57 determined by analyzing for phosphorus according to the method of Chen *et al.* (1956) with minor modifications. In this procedure the phosphorus-containing compound is oxidized by heating with several drops of concentrated sulfuric acid followed by treatment with 60% perchloric acid. A molybdenum blue color is developed by production of phosphomolybdenum complex, which is reduced with ascorbic acid. One microgram of phosphorus can readily be measured in a final volume of 5 ml. This is equivalent to 10.37 micrograms of ET-57. Results were found to be accurate to ±10%.

The entomological data are reported in table 1.

There was a 50-fold increase in the ET-57 concentration in air in the temperature interval 15° to 45° C., as shown below:

°C.	*Micrograms/liter*
15	0.03
24	.09
38	.50
42	1.2
45	1.5

The insecticidal effectiveness of the treatment also increased with increasing temperature (table 1).

All flies and mosquitoes exposed to ET-57 vapors for 1 hour at 25° to 27° C. were killed. A 4-hour exposure killed all flour beetles at 24° to 33° and all cockroaches at 33° to 46° C. A surprising observation was the greater resistance of mosquitoes as compared with flies.

The concentration of ET-57 in air at temperatures above 25° C. may be too high for practical use. Further tests will be conducted with this compound dissolved in a substance designed to reduce its vapor pressure.

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Host Relationships of Seed Chalcids Reared from Birdsfoot Trefoil[1]

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In a series of studies, in Russia, it has been shown that the chalcids which feed as larvae upon the seeds of various leguminous plants, and which have been collectively called clover seed chalcids (*Bruchophagus gibbus* (Boh.)), are actually a complex of morphologically distinct species. Host relationship studies with a few of these insects have demonstrated that, in general, each species appears to be restricted to a particular genus of plants, attacking several species within the genus. For example, *Bruchophagus roddi* Guss. has been reported as capable of infesting only the seeds of alfalfa (*Medicago sativa* L.), and *Medicago falcata* L., while *B. gibbus* (Boh.) has been reported as restricted to red clover (*Trifolium pratense* L.), and *Trifolium medium* L. (Kolobova 1930, Nikolskaja 1952, Fedoseeva 1954).

Hansen (1955) has demonstrated that clover seed chalcid populations associated with alfalfa and red clover in the United States are, also, each restricted to species of *Medicago* and *Trifolium*, respectively.

The present paper is concerned with a study of the host relationships of populations of seed chalcids reared from birdsfoot trefoil (*Lotus corniculatus* L.). This group of insects is, in all probability, the species *Bruchophagus kolobovae* Fed. recently described in Russia by Fedoseeva (1956). However, a comparison of North American seed chalcids reared from birdsfoot trefoil with European specimens of *B. kolobovae* has not as yet been attempted, and, therefore, a definite statement concerning the two populations is not possible at this time. Fedoseeva confined his studies on *B. kolobovae* to a consideration of the morphology of the species. No information has been published concerning the host relationships of seed chalcids associated with birdsfoot trefoil either in Europe or in this country.

MATERIALS AND METHODS.—Approximately 100 plants of alfalfa, red clover, and several species of *Lotus* were grown from seed or cuttings, and placed in clay pots outdoors. Shortly after the florets of each species blossomed and were pollinated, groups

[1] Accepted for publication March 3, 1958.

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Table 1.—The percentage of infested seed of leguminous plants after placing these plants in cages containing seed chalcids obtained from birdsfoot trefoil seed.

Plants Caged	Number of Replicates	Total Number of Seeds Developing	Number of Seeds Infested	Percentage Infestation
Lotus corniculatus	10	149	84	56.4
L. tenuis	5	74	12	16.2
L. uliginosus	10	494	247	50.1
L. hispidus	10	385	17	4.4
L. americanus	7	29	0	0.0
L. tetragonolobus	4	22	0	0.0
Medicago sativa	10	54	0	0.0
Trifolium pratense	9	272	0	0.0

Table 2.—The percentage of infested seed of leguminous plants after placing these plants in cages containing seed chalcids obtained from narrow-leaved trefoil seed.

Plants Caged	Number of Replicates	Total Number of Seeds Developing	Number of Seeds Infested	Percentage Infestation
Lotus corniculatus	3	15	9	60.0
L. tenuis	5	60	38	63.3
L. uliginosus	4	227	17	7.5
Medicago sativa	3	19	0	0.0
Trifolium pratense	4	86	0	0.0

of the tiny seed pods were enclosed in small glassine bags to prevent seed chalcid infestation in the field. With plants such as red clover and alfalfa, whose florets blossom over an extended period of time, the flower heads were bagged shortly after the first few florets were pollinated. Approximately 1 week after bagging, the closures were removed from the seed pods, and individual plants were placed in cages containing approximately 30 male and female seed chalcids reared from birdsfoot trefoil seed. After a period of one to several days, the plants were removed from the cages and again placed outdoors. The seed pods were tagged at this time, and once again enclosed in glassine bags. When the seed pods reached maturity, they were harvested and placed in muslin-capped glass vials, and held until time was available for their examination. In some instances, dissection of the seed was delayed for a number of months, and some calchids completed their development within the seed and emerged as adults.

RESULTS.—No seed chalcids were dissected or reared from alfalfa or red clover seed which had been caged during its development with seed chalcids obtained from birdsfoot trefoil,[3] indicating that the caged insects did not oviposit in the seeds of these species. The possibility that oviposition occurred, but that development was suppressed, was not supported by observations on the behavior of the female chalcids, and the dissection of a number of seeds shortly after caging them with the insects.

Of the *Lotus* species tested, the caged chalcids oviposited in the seeds of birdsfoot trefoil, narrow-leaved trefoil (*L. tenuis* Waldst. & Kit.), *L. uliginosus* Schk., and *L. hispidus* Desf. However, no seed chalcids were dissected or reared from the seeds of *L. americanus* (Nutt.) Bisch. or *L. tetragonolobus* L. (Table 1).

A considerable range in size of seed exists among the *Lotus* species used in the studies. Seed chalcid infestation was limited to those species having seed approximately the same size or smaller than birdsfoot trefoil seed. Some adults emerged from the seeds of *L. uliginosus* which are on the average only slightly larger than one-half the size of birdsfoot trefoil seed.

Subsequent to these tests, investigations were made by the senior author of naturalized stands of narrow-leaved trefoil which occurs in eastern New York. Seed samples collected from these plants were found to be heavily infested with seed chalcids. Cage studies conducted with these insects substantiated the earlier findings obtained with individuals from birdsfoot trefoil (table 2).

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[3] Seed chalcids reared from alfalfa and red clover seed and caged with alfalfa and red clover plants bearing seed pods, in a state of development comparable to those offered to seed chalcids reared from birdsfoot trefoil, readily accepted the developing seeds as oviposition sites, and a number of chalcids were reared through to adults from this material.

Variegated Cutworm Injury to Ripe Strawberries[1]

E. R. OATMAN, *University of Wisconsin, Madison*

A strawberry grower in the vicinity of Sturgeon Bay visited the Peninsula Branch Experiment Station in July of 1957 with a sample of ripe strawberries of the Robinson variety. The berries, which were from the first picking, appeared to be injured by chewing insects. The injured areas ranged from $\frac{1}{8}$ to $\frac{1}{4}$ inch both in depth and width.

It was found that a high population of cutworm larvae was responsible for the injury. Some of the larvae were observed feeding on the berries, but most were found curled on the surface of the mulch and duff at the base of the strawberry plants where they had dropped when disturbed. Many of the larvae were in the last larval instar. The region of heaviest infestation, covering approximately 1 acre, was located at the north and lower end of the field. This area had a fairly heavy growth of oats which had grown in the rows as a result of an oat straw mulch applied the previous fall.

The larvae were tentatively identified as the variegated cutworm, *Peridroma margaritosa* (Haw.). Figure 1 shows the cutworms and their feeding injury on strawberry.

A thorough spray of malathion at 1 pound actual per acre was immediately applied by the grower without any appreciable effect. Field collected larvae were then laboratory treated with an excess dosage of 1% rotenone dust and confined in a 1-gallon ice cream carton containing similarly treated strawberry fruit and foliage. This treatment was also without effect and after 7 to 10 days the larvae completed their development and pupated.

One of the adults, which later emerged, was forwarded to the Insect Identification and Parasite Introduction Laboratories,

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station, Accepted for publication March 3, 1958.

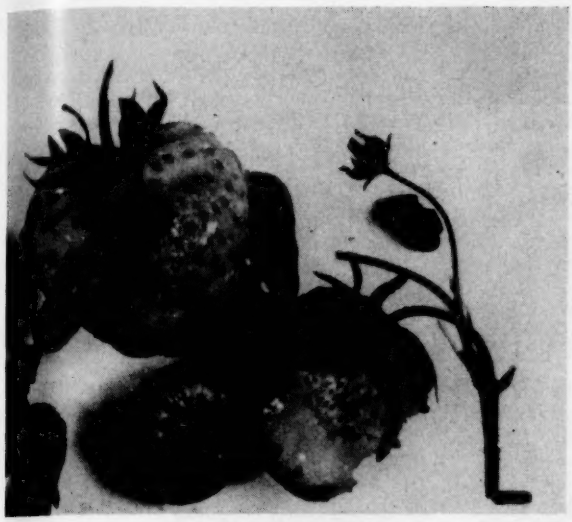

FIG. 1.—Feeding injury on strawberry fruit by larvae of the variegated cutworm, *Peridroma margaritosa* (Haw.).

U. S. Department of Agriculture, Beltsville, Maryland, where the determination was confirmed by Dr. E. L. Todd.

The strawberries in the infested area of the field were so severely injured that it was not economical to harvest the crop.

Control of Paddy Borer on the Second Rice Crop in 1957[1]

CHIA-HWA TAO, *Chia-yi Agricultural Experiment Station, Taiwan, China*

The paddy borer (*Schoenobius incertulas* Wlk.) has been a serious pest of the rice crops in the Southern part of Taiwan. It has five or six generations a year. The second rice crop is destroyed by the fourth and fifth generations. They attack this crop from the seedling stage to maturity. Heavy infestation of paddy borer in the fields usually occurs every year. Recent tests by the writer from 1953 to 1956 have given hope for controlling this pest with certain insecticides. During the summer of 1957 the writer was provided an opportunity to retest some of these insecticides as well as to use some untried materials for paddy borer control. The results obtained in these tests are summarized herein.

METHODS.—The field trials were carried out at Shang-tzu-ting in Chia-yi County in 1957. The field experiments were divided into 4 blocks of 12 plots each. Plots measured 5×5 meters including 500 hills, each plot had 20 rows making 25 hills per row. The plants were spaced 25 cm. by 20 cm. apart. The variety of rice used was Chia-yoh No. 242. It was transplanted on August 3. Ten different treatments and two untreated checks were assigned at random to each block. The plots were sprayed with emulsions of 19.5% endrin, 20% Guthion, 46.6% Folidol (46.6% active ingredients of Diethyl-p-nitrophenyl thiophosphoric ester), 37% Trithion and 48% methyl parathion. These concentrations were based on applying the active materials at 1.411 lbs. or 2.822 lbs. per hectare. Three applications were made. The percentage of dead seedlings was determined by examining 150 hills randomly selected in each plot 7 days after first spraying and the percentage of empty panicles or white heads was determined by examining 150 hills at random in each plot 2 weeks before harvest.

Sprays were made from emulsifiable concentrates and applied with a small type of automatic sprayer. Applications of insecticides were made on Sept. 6, since the fourth generation of this borer attacked seedlings during the growing stage. The rate of application was 800 liters per hectare and the first application was followed by a second on Sept. 30 and a third on Oct. 5. Since the fifth generation of the borer started to attack the boot stage or youngest panicle stage, the rate of application was increased to 1200 liters of solution per hectare for each of the last two treatments.

Table 1.—Control of paddy borer on the second rice crop with emulsions of insecticides in 1957.

Emulsion Used	Dead Seedlings		Empty Panicles		Yield of Rice Kg./Ha.	Index
	Per Cent	Angle	Per Cent	Angle		
0.02% Endrin	2.8	9.65	7.3	15.85	5,280.0	120.8
0.04% Endrin	1.8	7.52	3.5	10.49	6,040.0	138.2
0.02% Folidol	2.5	8.97	9.3	17.71	5,080.0	116.3
0.04% Folidol	1.8	7.45	5.2	12.59	5,720.0	130.0
0.02% Guthion	2.8	9.64	6.3	14.57	5,360.0	122.7
0.04% Guthion	1.5	6.90	5.6	13.58	5,800.0	132.7
0.02% Trithion	3.6	10.95	8.2	16.49	5,200.0	119.0
0.04% Trithion	1.4	6.64	7.4	15.54	5,440.0	124.5
0.02% Methyl parathion	4.1	11.60	9.8	18.17	4,840.0	110.8
0.04% Methyl parathion	2.8	9.52	8.0	16.49	5,160.0	118.1
Check (untreated)	6.3	14.50	11.9	20.72	4,370.0	100.0
L.S.D. at 5%-level		2.08		3.15	34'.7	
1%-level		2.79		4.23	459.8	

RESULTS.—All treatments showed significant differences in yield of rice over the control. There were also significant differences between the insecticides and the two concentrations used. According to the yields from treated plots, endrin, Guthion and Folidol were about equally effective, giving excellent control of this pest. Endrin and Guthion were better than Folidol and Trithion and methyl parathion were the least effective treatments, as shown in table 1.

DISCUSSION.—The percentage of dead seedlings in the treated plots was 1.4 to 4.1 as compared with 6.3 in the check. The percentage of empty panicles or white heads was 3.5 to 9.8 in the treated plots as compared with 11.9 in the untreated plots. And the yields from treated plots were 4840 to 6040 kgs. of rice per hectare as compared with 4370 kgs. in the check or an increase of 470 to 1670 kgs. per hectare. Although all treated plots were better than the untreated check, the 0.04% formulations gave better results than the 0.02% concentrations. Endrin and Guthion were found to be most effective in controlling this pest. In all treated plots the 0.04% concentrations were about equally effective against this pest except Trithion and methyl parathion. These were not as effective as the others, but Trithion was better than methyl parathion.

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Suppression of House Fly Breeding in Tomato Cannery Waste Lagoons by Means of Polyethylene Cover[1]

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From the standpoint of public health, the house fly, (*Musca domestica* L.) is the outstanding insect problem encountered by the small, independently-owned tomato packing plants in Indiana. This problem stems largely from the customary practice of handling the cannery wastes in a lagoon. In most cases, these plants are located in small rural communities where modern sewage treating facilities are unavailable. The cannery waste lagoon, with its large quantities of decaying organic matter, is the breeding site for tremendous populations of flies. Maximum fly populations occur simultaneously with the tomato packing

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FIG. 1.—A polyethylene cover installed over the waste lagoon of a tomato cannery.

season which normally runs from late August to early October.

Attempts to combat this situation with insecticides have not been successful because of the difficulties involved in application. The introduction of a toxicant into the sewage stream as it leaves the plant is impractical because of the severe dilution which occurs in the lagoon. Topical application to the organic conglomerate is likewise impractical because this surface continually cracks open, exposing untreated material to ovipositing flies.

The wide range of uses to which large plastic sheets have been put suggested the possibility of controlling fly breeding in waste lagoons by covering them with such a plastic film. Immediately prior to the start of the 1957 packing season, the lagoon of the Eaton Canning Company, Eaton, Indiana (R. M. Butterfield, owner-manager) was covered with a black polyethylene sheet, a product of the Visking Company, Terre Haute, Ind. The wastes were piped into the lagoon under the cover. Sufficient slack was provided in the cover to allow it to move up and down with the level of the water and wastes in the lagoon.

The lagoon was 90 feet wide by 140 feet long. The cover was 100 feet wide by 144 feet long. It was made by joining six strips each of which were 24 feet by 100 feet. The strips were joined by a hand operated band-type heat sealer such as is commonly used to seal small plastic food packages. This required a great deal of time and labor. Fabrication of such a cover surely could be done more satisfactorily by the plastic manufacturer in the case of commercial installation.

The cover weighed approximately 475 pounds. However, when spread out in a single thickness it was light enough to be airborne. It was easily installed on the lagoon as four men carried the leading edge into a light breeze. The weight of the cover was supported by the air trapped under it. The edges were then secured in place along the top of the lagoon dike by a covering of earth (fig. 1). Installation required less than one-half day.

Despite the best efforts of the management of this cannery the lagoon had consistently been the source of fly populations which were objectionable not only in the immediate vicinity of the plant but over the entire community. At the height of the 1957 packing season flies were so scarce that none came to rest on a Scudder grill placed on the cannery loading dock. Other plants in the state were experiencing normal fly problems at this time. No flies were observed around the lagoon throughout the season and the plastic cover was credited with complete suppression of fly breeding at this site.

Although rainfall was considerably above normal for this time of year in 1957, at no time did sufficient water collect on top of the cover to cause it to submerge. Gaseous fermentation products did not collect underneath the cover in sufficient amounts to cause it to balloon. The adhesion between the cover and the liquid surface it rested upon was more than adequate to prevent wind from damaging the covery by causing it to flap. The cover did not aggravate the problems presented by the evolution of unpleasant odors from the lagoon.

Polyethylene is noted for its resistance to chemicals. The useful life of the cover is unknown at present but at least 2 years of service is anticipated.

The cost of the cover, including installation labor, was approximately $500.00. Figuring the life of the cover at 2 years the annual cost for fly control would be $250.00. The material needed for 8 weekly applications of a larvicide would be approximately $100.00. Labor and maintenance of equipment would probably be an equal amount. The superior results obtained with the plastic cover expressed in terms of fewer residual applications at the plant should make fly control by means of such a cover actually cheaper than with chemicals alone.

Control of Mites on Hamsters[1]

HAROLD GEORGE SCOTT[2]

Large numbers of mites on golden hamsters (*Mesocricetus auratus*) were reported at a Communicable Disease Center research laboratory at Chamblee, Georgia, in June 1956. An investi-

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gation revealed that the animals were infested, at an estimated 50 mites per animal, with *Ornithonyssus bacoti* Hirst, the tropical rat mite.

Pesticides which do not obviously harm experimental animals can nonetheless modify experimental data (Lehman & Moskey 1951). Yet commonly recommended control techniques require intimate contact between the experimental animals and an insecticide. Olson & Dahms (1946) applied 10% DDT dust to animal bedding and animals, and applied 5% DDT solution to the walls of the animal houses. Freshly washed cages were steamed. Fisk (1951) dusted laboratory mice with 10% ovex (formerly called Ovotran) and dipped freshly washed cages in 1% cresols emulsion ("Lysol").

As a long-term experiment was in progress at the Chamblee laboratory, nothing which would upset the physiology of the hamsters could be used. The removable tray at the bottom of each cage was painted with an emulsion containing 2% chlordane, and the chemical was renewed each time the cages were washed. No physiologic change associated with the chlordane was detected. The mite population was reduced to approximately three mites per animal within 2 weeks. To control the mites remaining, painting with chlordane was continued and the cages were sprayed after each washing with 0.3% pyrethrins —1.5% piperonyl butoxide solution. Animals not involved in experiments were dusted with 0.11% lindane—1.91% methoxychlor which efficiently eliminated the mites. No attempt was made to determine if residual damage occurred as a result of the use of this dust. Control has been maintained using these techniques.

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The Toxicities of Fluorinated Cyclohexanes to House Flies[1]

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The isomers of benzene hexachloride (BHC) illustrate an important example of the critical relationship between stereochemistry and biological activity. Of the six isomers of BHC evaluated as insecticides only the gamma isomer has shown appreciable activity (Metcalf 1947, Back 1951). The inactivity of hexaethylcyclohexane (Stringer & Woodcock 1948) and hexamethoxycyclohexane (McGowen 1947) further suggest a rigid relationship between constitution and activity associated with the gamma isomer of BHC. Fluorinated analogs of BHC have received little attention because of the unavailability of these compounds in the past. However, the gas chromatographic technique of Godsell *et al.* (1956) has made available a large series of fluorocyclohexanes. This paper describes the evaluation of several of these compounds as insecticides against the house fly (*Musca domestica* L.). In addition, two of the compounds were evaluated as lindane synergists in a strain of lindane resistant house flies.

Materials and Procedure.—The fluorocyclohexanes are listed in table 1. All materials were pure and were prepared as acetone solutions for house fly treatment. Female house flies, reared according to methods of the Chemical Specialties Manufacturers' Association and 3 to 4 days of age, were used in each test. The compounds were administered in one μl. of acetone by a micrometer-driven syringe. Each fly was treated with a 10-μg. dosage and if mortality was negligible at this dosage the compound was regarded as insecticidally inactive. After treatment the flies were given a milk solution and held at approximately 27° C. Mortality counts were made 24 hours after treatment.

The ability of two compounds, polyfluoro BHC analogs, to synergize lindane in resistant house flies[3] was determined by treating flies with a 1:1 mixture of lindane and the analog. Four dosages were evaluated, 10:10, 5:5, 2.5:2.5 and 1.25:1.25 (μg:μg). Treatment and holding conditions were the same as described above. The LD-50 of lindane for these flies was greater than 50 μg.

Results and Discussion.—None of the compounds evaluated exhibited appreciable activity (table 1). In addition, hexachloropentafluorocyclohexane (1) and hexachlorohexafluorocyclohexane (2) were inactive as synergists for lindane in resistant house flies.

The unavailability of hexafluorocyclohexane, the fluoro analog of BHC, militates against drawing general structural-activity relationships among the fluorocyclohexanes. However, certain comparisons can be made between analogs of BHC and some of their fluorinated counterparts because of their close halogenic relationship. The inactivity of the hepta-, octa-, and nonafluorocyclohexanes compares with the corresponding polychloro compounds (Metcalf 1947, Riemschneider 1950).

The inactivities of hexachloropentafluorocyclohexane and hexachlorohexafluorocyclohexane are interesting. These polyfluoro analogs of BHC should be closely isosteric with BHC. However, the isomeric composition of the polyfluorinated hexachlorocyclohexanes may not necessarily be the same as that of BHC which derives its toxicity primarily from one isomer, the gamma component. This fact alone could serve to diminish the toxicity of the fluorinated cyclohexanes. Riemschnedier (1950) prepared and evaluated hexachloromonofluorocyclohexane against house flies and found the isomeric mixture to be about one-thirteenth as toxic as lindane. Since Riemschneider had demonstrated that the active constituent in the monofluoro BHC mixture represents only 15% of the total mixture, the toxicity of the active component is actually almost half that of its corresponding BHC isomer, lindane. The composition of the monofluoro BHC isomeric mixture probably resembles that of BHC, since the percentage of component corresponding to lindane is about the same as that reported for lindane in BHC mixtures (Ramsey & Patterson 1946). Whereas monofluorination reduces lindane toxicity slightly, penta- and hexafluorination completely destroy the insecticidal activity of the isomeric mixture. The complete loss of activity associated with the polyfluorination of BHC probably indicates that the isomeric composition differs from that of lindane.

Moorefield & Kearns (1955) have demonstrated that DDT synergists act by inhibiting the enzyme responsible for DDT degradation. Since fluorinated analogs of DDT inhibit DDT breakdown (Cohen & Tahori 1957), it seems possible that fluorinated analogs of lindane could act similarly to inhibit lindane detoxication. Compounds which are isosteric with lindane could conceivably inhibit the initial step in detoxication, dehydrochlorination (Sternburg & Kearns 1956), if the mechanism is similar to the dehydrohalogenation of DDT (Sternburg & Kearns 1950). The inactivity of penta- and hexachlorohexafluorocyclohexane as synergists for lindane against house flies may further reflect stereochemical differences between these compounds and lindane. If lindane synergists (of which none are known) have the same requirements as those of DDT, then the

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[2] The author wishes to thank Dr. J. C. Tatlow, the University, Edgbaston, Birmingham, England and Dr. J. Traynham, Louisiana State University, for their interest and discussions of this work.

[3] The author wishes to thank Dr. A. S. Perry, U. S. Public Health Service, Savannah, Georgia, for supplying this strain of house flies.

Table 1.—Chemical data and toxicities of eight fluorocyclohexanes topically applied (10 μg./fly) to susceptible house flies.

Compound[a]			Per Cent Mortality (24-Hour)
Empirical Formula	Name[b]	b.p. (° C.)	
$C_6HCl_6F_5$	1,2,3,4,5,6-hexachloropentafluorocyclohexane	92°/0.5 mm.	0
$C_6Cl_6F_6$	1,2,3,4,5,6-hexachlorohexafluorocyclohexane	137°/30 mm. (m.p. 101–102°)	0
$C_6H_5F_7$	1H,2H,4H-heptafluorocyclohex-1-ene	106°	0
$C_6H_4F_8$	1Ha,2Ha,4He,5He-octafluorocyclohexane	118°	20
$C_6H_2F_8$	1H,4H-octafluorocyclohex-1-ene	84°	0
$C_6H_3F_9$	1Ha,2Ha,4Ha-nonafluorocyclohexane	101° (m.p. 12–13°)	0
$C_6H_3F_9$	1Ha,2Ha,4He-nonafluorocyclohexane	92° (m.p. 44°)	0
$C_6H_3F_9$	1Ha,2He,4Ha-nonafluorocyclohexane	107°	10

[a] Obtained through the courtesy of J. C. Tatlow. The University, Edgbaston, Birmingham, England.
[b] The designation of a (axial) and e (equatorial) indicates stereochemical configurations.

inactivity of these polyfluoro BHC analogs is probably due to stereochemical factors.

That the fluorinated analogs of hexachlorocyclohexane are inactive due to detoxication seems unlikely. If the mechanism of detoxication resembled that of lindane, a trans elimination would occur resulting in a possible elimination of fluorine (Tatlow 1958). This enzymatic reaction certainly would be more difficult than simple dehydrochlorination which is not highly developed in flies until after intensive selection pressure by lindane.

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Susceptibilities of Four Species of Potato-Infesting Aphids to Insecticides[1]

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In the commercial potato-growing areas of New Brunswick, DDT has given very satisfactory control of aphids on potatoes. However, in certain coastal areas and in some other provinces it has been much less satisfactory.

Four potato-infesting species of aphids are commonly found in most potato-growing areas of North America, but the numbers of each species vary considerably from district to district and from week to week each season. Previous investigations by Adams (1950), Campbell *et al.* (1948), Fernow *et al.* (1953), Gibson *et al.* (1951), and Shands *et al.* (1950) have either disregarded or attributed little importance to the species of aphids present. However, differences in relative numbers of the species might well account for the conflicting reports on the results obtained with DDT. Accordingly, an experiment was designed to determine, under controlled conditions in the greenhouse, the effectiveness of a few insecticides against the four potato-infesting species: the green peach aphid, *Myzus persicae* (Sulz.); the potato aphid, *Macrosiphum solanifolii* (Ashm.); the buckthorn aphid, *Aphis abbreviata* Patch; and the foxglove aphid, *Myzus solani* (Kltb.).

Materials and Methods.—In 1954 the four species were reared throughout the year in the greenhouse by MacGillivray (1955) for the first time. The same author (1958) has shown that the mean age at which adults of the four species began to reproduce ranged from 8.69 to 11.74 days. Hence, 9-day-old wingless adults were used.

Healthy Katahdin potato leaves were used as the spray surface. The leaves were held in small vials filled with water, the vials being set in vermiculite in 6-inch clay pots. The insecticides were applied in a fume cabinet with an atomizer. Pressure was maintained at 5.5 inches of mercury and leaves were sprayed to point of run-off. The leaves were allowed to dry 2 hours, when they were placed in individual cages and 5 aphids were placed on each leaf.

There were six replicates for each treatment with each species. Each cage was examined every 24 hours. Whenever no living aphids were present, the leaf was re-infested with five aphids of the appropriate species. The aphids were considered established and not affected by the insecticide when they began producing nymphs.

Because of limitation on space the experiment was run an average of 7.5, 8.5, 13.5, and 15.0 days, respectively, for *Myzus persicae*, *Macrosiphum solanifolii*, *Myzus solani*, and *Aphis abbreviata*.

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[2] Associate Entomologist, Field Crop Insect Section.

RESULTS.—Table 1 clearly indicates a distinct species reaction to each insecticide, and at no time were the leaves free of *M. solanifolii* when DDT was used.

Table 1.—Average number of days before aphids of four species became established on potato leaves in the laboratory after treatment with various insecticides.

INSECTICIDE	FORMULATION	POUNDS PER 100 GAL.	*Myzus persicae*	*Macrosiphum solanifolii*	*Myzus solani*	*Aphis abbreviata*
Malathion	25% w.p.	2.5	7.5+	8.5+	13.5+	15.0+
DDT	20% emul.	5	4.6	1.3	11.5	4.0
Toxaphene	40% w.p.	2.5	6.5	4.6	3.8	4.3
Lindane	20% emul.	0.625	4.6	2.0	5.0	4.5
Toxaphene+DDT	40% w.p.+	2.5+	5.6	3.3	11.0	8.2
	20% emul.	5				

The analysis of the number of living aphids found 24 hours after each re-infestation showed that for *Myzus persicae* and *M. solani* there was no significant difference between malathion, DDT, and toxaphene plus DDT. These chemicals were all more toxic than lindane or toxaphene at the 1% level of significance. Lindane was more toxic than toxaphene at the 5% level of significance.

For *Macrosiphum solanifolii* malathion was more toxic than all the other treatments at the 1% level of significance. Lindane was more toxic than DDT, toxaphene, and toxaphene plus DDT at the 1% level of significance. Toxaphene and toxaphene plus DDT were more toxic than DDT at the 5% level of significance.

With *A. abbreviata* malathion was more toxic than all the other treatments at the 1% level of significance. The remaining treatments followed each other in the following order: Toxaphene plus DDT, DDT, lindane, and toxaphene each being more toxic than those following it at the 5% level of significance.

This experiment indicates that a chemical lethal to one species of potato-infesting aphid may or may not be of service in limiting other species in a heterogeneous population.

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OBITUARY

Joseph M. Davis
1909-1957

Joseph M. Davis, Entomologist of the Forest Insect Laboratory, Forest Service, Beltsville, Maryland, died on December 30, 1957 following an illness of several months.

Mr. Davis, the son of Charles W. and Margaret (Puhr) Davis, was born on May 17, 1909 at Noonan, North Dakota. He grew up there on the family farm and then attended the University of North Dakota where he received a B.S. degree in 1935 and an M.S. degree in 1937. Later, he took additional graduate work at the University of Wisconsin and the University of Utah. Although his training was primarily in entomology and the biological sciences, he also acquired a considerable background in mathematics, physics, and chemistry.

After receiving his Master's degree, Mr. Davis was appointed an instructor at the North Dakota School of Forestry where he taught courses in mathematics and physics. He left there in 1941 to accept a position as research biologist with the North Dakota Game and Fish Department.

In 1942, he came to the National Bureau of Standards, Washington, D.C., as a research physicist, working on special aviation equipment for the Armed Forces. When the war ended, he was engaged for a short while in federal plant quarantine work in Washington.

In 1946, Mr. Davis joined the Forest Insect Laboratory at the Beltsville Research Center as a research entomologist. His principal fields of investigation were in aerial spraying for controlling forest defoliators, particularly studies on spray distribution and atomization. He also pioneered in the use of radioisotopes for studying the dispersal habits of various forest insects. He published several papers on these subjects.

He was a member of the American Association for the Advancement of Science, the Entomological Society of America, the Entomological Society of Washington, the Insecticide Society of Washington, Phi Delta Kappa honorary education society, Sigma Xi, and the Masonic Order.

He is survived by his wife, the former Miss Evelyn Johnson of Fargo, North Dakota, whom he married in 1941, and by three sons: Bryan 15, Allan 12 and Darrell 9 years old.

In many respects, Joe Davis was an unusual man. Probably his most outstanding trait was his broad range of interests and abilities. Not only being a well rounded entomologist, but also having a strong bent for mathematics, he approached a research problem with the keen analysis of a mathematical mind. He was a stimulating idea man, a skilled technician, and a leader of team research. In addition to his profession, Davis had several avocations. Over a period of years, he built a spider collection, numbering some 3700 specimens, which he presented to the Smithsonian Institution. He had a great love for music, and not only played the clarinet and viola, but at one time was director of the civic orchestra in Bottineau, North Dakota. He also was highly skilled in photography, and liked to take both still and motion pictures of many of the insects he encountered; such as walkingsticks, pine sawflies, and the spruce budworm. Being especially ingenious, he built a special box for field use in taking pictures of insects which approximated dark room conditions. This was only one example of several pieces of equipment he made to facilitate his research work in the field of forest insects.

In his death, his profession lost an exceptional scientist; his associates, a highly valued friend.

R. A. ST. GEORGE
J. S. YUILL

BOOK REVIEW

ANNUAL REVIEW OF ENTOMOLOGY, VOL. 3, 1958. Editor, Edward A. Steinhaus, University of California, Associate Editor, Ray F. Smith, University of California. 8vo., cloth, 520 pp., Palo Alto, California, Annual Reviews, Inc., 1958. $7.00.

This volume is one of a series in which specialists in the various branches of entomology have prepared for their colleagues authoritative and scholarly reviews of their various specialties. It is certain that this will be given the same warm welcome accorded those previously issued because it possesses the same outstanding usefulness. The very definite need for a reference work of this particular type was long realized by all of us who had to struggle with the widely scattered literature of entomology. There had not existed anywhere a work of its exact scope. So, in 1953, a Committee of the Entomological Society of America was appointed to examine the problem of providing adequate reviews of entomological literature, and, after exhaustive study, it recommended that such needs probably would be best met by a review publication of the general type of those published by the nonprofit organization, Annual Reviews, Inc., which since 1931, has devoted itself to the publication year by year of critical reviews designed to cover systematically the current literature in certain major fields of science, in which the problems were similar to those of the entomologists. After appropriate investigations and appraisal the work was started in 1956 cooperatively between Annual Reviews, Inc., and the Entomological Society of America. The main objective was publication of authoritative and concise treatments of definitive subjects of current interest. As would be expected the more active fields of research require critical reviews annually, while the less active need to be summarized and evaluated as developments require. Also, by presenting divergent viewpoints in successive years, well rounded treatment of a given subject eventually should be attained. Now that a three-year start has been made—now that the ice definitely has been broken—it is easy to visualize that each succeeding volume of this series will average up gradually better in every way than those before. Minor flaws will be eliminated, the scope of the plan as a whole will gain steadily in practical utility and in wider usefulness.

A review of Volume 1, of this series was published in the *Bulletin of the Entomological Society of America*, 2: 27–29, 1956, and of Volume 2, in the JOURNAL OF ECONOMIC ENTOMOLOGY, 50: 377, 1957. Some idea of the scope, the subjects, and the selection of authorships may be gained by enumeration of the 23 papers making up the newly issued Volume 3 of the series. These are as follows: "The Nervous System," by K. D. Roeder, Tufts University, Medford, Mass. 18 pp., 105 refs.; "Chemoreception in Arthropods," by E. S. Hodgson, Columbia University, N. Y. 18 pp., illus., 75 refs.; "Internal Symbiosis in Insects," by A. G. Richards and M. A. Brooks, University of Minnesota, St. Paul. 20 pp., 83 refs.; "Nutritional Requirements of Phytophagous Insects," by W. G. Friend, Canada Department of Agriculture, Ottawa, Ontario. 18 pp., 118 refs.; "Recent Advances in Silkworm Nutrition," by J. M. Legay, Institut National de la Recherche Agronomique, France. 12 pp., 84 refs.; "Uses of Sounds by Insects," by H. Frings and M. Frings, Pennsylvania State University, University Park, Pa., 19 pp., 137 refs.; "Dynamics of Insect Populations," by A. J. Nicholson, Commonwealth Research Organization, Canberra, Australia. 29 pp., 27 refs.; "Ovarian Structure and Vitellogenesis in Insects," by P. F. Bonhag, University of California, Berkeley. 23 pp., illus., 104 refs.; "Genetics and Breeding of the Honey Bee," by W. C. Rothenbuhler, Iowa State College, Ames. 19 pp., illus., 129 refs.; "Phylogeny of the Panorpoid Orders," by H. E. Hinton, University of Bristol, England. 25 pp., 64 refs.; "Zoogeography of Insects," by J. L. Gressitt, Bishop Museum, Honolulu, Hawaii. 24 pp., 209 refs.; "Hybridization and Speciation in Mosquitoes," by L. E. Rozeboom, Johns Hopkins University, Baltimore, and J. B. Kitzmiller, University of Illinois, Urbana. 18 pp., 64 refs.; "Feeding Habits of Biting Flies and Their Significance in Classification," by J. A. Downes, Department of Agriculture, Ottawa, Canada. 18 pp., 139 refs.; "Resistance of Plants to Insects," by R. H. Painter, Kansas State College, Manhattan. 34 pp., 198 refs.; "Biological Control of Insect Pests," by C. P. Clausen, University of California, Riverside. 29 pp., 119 refs.; "Biology of Scarabaeidae," by P. O. Ritcher, Oregon State College, Corvallis. 23 pp., 174 refs.; "Insect Eradication Programs," by W. L. Popham and D. G. Hall, U. S. Department of Agriculture. 19 pp., illus., 9 refs.; "Chemistry and Action of Acaricides," by R. B. March, University of California, Riverside. 21 pp., 151 refs.; "Organic Phosphorus Insecticides for Control of Field Crop Insects," by W. A. L. David, Agricultural Research Council, Cambridge, England. 23 pp., 184 refs.; "Insecticides for Control of Adult Diptera," by R. W. Fay and J. W. Kilpatrick, U. S. Public Health Service, Savannah, Georgia. 19 pp., 153 refs.; "Forage Insects and Their Control," by G. G. Gyrisco, Cornell University, Ithaca, N. Y. 27 pp., 285 refs.; "Control of Forest Insects," by R. E. Balch, Forest Biology Laboratory, New Brunswick, Canada. 19 pp., 134 refs; and "Transmission of Plant Viruses by Arthropods," by K. M. Smith, Agricultural Research Council, Cambridge, England. 13 pp., 62 refs.

It will be noted that each paper has been accompanied by an appropriate bibliography (but regrettably with titles of many cited references omitted), these varying from 9 to 285 titles each, with a total of 2807 or an average of 122 titles. Author and subject indexes to the entire volume, making an additional 37 pages, also are included. It is regretted that space limitations here forbid even brief discussion of each of the 23 papers making up this book. It is, however, perhaps enough to state that on the whole, this, in all probability, along with the other two volumes of the series, previously issued, makes up one of the most important and certainly one of the most useful general reference works to appear in a long time. It is a pleasure to commend this latest volume to the attention of entomological fellow workers everywhere. The series "will be extremely helpful in improving the position of entomology as a growing science of the first rank."

J. S. WADE

NOTICE TO AUTHORS

Authors are urged to consult the Publication Policies and Manuscript Rules and Suggestions (Vol. 49(4): 575–7) before preparing manuscripts intended for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY.